JOURNAL OF ANIMAL SCIENCE

Published Quarterly

by the

American Society of Animal Production

MAY

1957

Volume 16 Number 2

EDITORIAL BOARD

J. K. Loosli, *Editor and Chairman*
W. D. Gallup, *Associate Editor*

O. G. Bentley (1959)*
L. J. Bratzler (1960)
A. B. Chapman (1958)
L. E. Hanson (1959)
C. F. Huffman (1958)
G. P. Lofgreen (1960)
A. V. Nalbandov (1957)
C. E. Terrill (1957)
S. W. Terrill (1958)

H. H. Stonaker, *Business Manager*

* Terms expire on December 31 of years indicated.

OFFICERS OF THE SOCIETY

J. C. Miller, *President*
W. M. Beeson, *Vice President*
H. H. Stonaker, *Secretary-Treasurer*
George Vander Noot, *North-Atlantic Vice President*
W. P. Garrigus, *Southern Vice President*
N. W. Hilston, *Western Vice President*
L. E. Casida, *Director*

Reprints of papers published herein are obtainable only from the authors.

Entered as second class matter under act of March 3, 1879, The Journal of Animal Science is published during February, May, August, and November by the Boyd Printing Company, Inc., 372–374 Broadway, Albany 7, N. Y. For Volume 16 (1957) domestic subscription price is $6.00; rest of North and South America is $6.50; and other places $7.00. Send your subscription to the publishers, 372–374 Broadway, Albany 7, New York or to H. H. Stonaker, Department of Animal Husbandry, Colorado A&M College, Fort Collins, Colo. Make check payable to the American Society of Animal Production. Foreign subscribers please remit by International money order or by a draft on a New York Bank. Notices of change of address should be in the hands of Dr. Stonaker 40 days previous to month of issue, to insure prompt delivery. Missing copies, owing to change in address cannot be supplied unless adequate notice has been given. Subscribers or members failing to receive a number of the Journal should notify the Business Manager within 90 days of publishing date. Missing copies will be replaced only if due to error on the part of the Business Manager or printing company.

Journal of

ANIMAL SCIENCE

VOLUME 16 MAY, 1957 NUMBER 2

CONTENTS

Page

The Effect of Steroid Hormones on Fattening Lambs. H. A. Henneman, R. E. Rust and J. Meites 283

Growth and Fattening Stimulation in Ewe Lambs by Certain Androgenic and Estrogenic Compounds. Bruce Taylor, W. H. Hale and Wise Burroughs 294

Estrogenic Activity of Feces and Urine Following Oral Administration of Diethylstilbestrol to Lambs. C. D. Story, E. W. Cheng, John Pauls and W. H. Hale 307

Observations on Parathyroidectomized Goats. V. R. Smith, G. H. Stott and C. W. Walker 312

Factors Affecting the Thyroid Uptake of I^{131} in Dairy Cows. E. W. Swanson, F. W. Lengemann and R. A. Monroe 318

Effect of Exposure to High Environmental Temperature and Shearing on Semen Production of Rams in Winter. R. H. Dutt and Paul T. Hamm 328

Ovulation, Prenatal Mortality and Litter Size in Swine. Earl L. Lasley 335

The Incidence of Extramedullary Myelopoiesis in Bovine Adrenal Glands. J. S. Greenstein and R. C. Foley 341

The Relative Emphasis Placed on Components of a Selection Index Under Two Nutritional Environments. R. E. Christian 349

The Value of Various Blends of Soybean Oil Meal, Peanut Oil Meal, and Degossypolized Cottonseed Oil Meal as Protein Supplements in Swine Rations. R. F. Sewell, B. C. Keen and J. L. Carmon 357

Effect of Protein Level in the Ration on Gossypol Tolerance in Growing Fattening Pigs. Fred Hale and Carl M. Lyman 364

The Effect of Amylases on the Digestibility of Starch by Baby Pigs. H. M. Cunningham and G. J. Brisson 370

The Relation Between Certain Characteristics of Fats and Oils and Their Apparent Digestibility by Young Pigs, Young Guinea Pigs and Pups. L. E. Lloyd and E. W. Crampton 377

The Digestibility of Ration Nutrients by Three- vs. Seven-week Old Pigs. L. E. Lloyd, E. W. Crampton and V. G. MacKay 383

Protein Requirements for Pigs Weaned at Two Weeks of Age. A. H. Jensen, D. E. Becker, H. W. Norton and S. W. Terrill 389

Page

Effects of Dietary Pantothenic Acid Levels on the Weanling Pig. C. E. Barnhart, D. V. Catron, G. C. Ashton and L. Y. Quinn... 396

High Levels of Dried Whey Powder in the Diet of Swine. D. E. Becker, S. W. Terrill, A. H. Jensen and L. J. Hanson..... 404

The Value of Fortified Creep Rations for Single and Twin Suckling Lambs. T. W. Perry, W. M. Beeson and Claude Harper..... 413

The Utilization of Ammoniated Cane Molasses by Sheep. Allen D. Tillman, Willis D. Gallup and Walter Woods..... 419

Some Effects on Beef Cattle of Protein and Energy Restriction. C. F. Winchester, R. L. Hiner and V. C. Scarborough..... 426

The Biological Availability of Calcium in Feeds for Cattle. S. L. Hansard, H. M. Crowder and W. A. Lyke..... 437

Availability of Phosphorus in Mineral Supplements for Beef Cattle. T. A. Long, A. D. Tillman, A. B. Nelson, W. D. Gallup and Bill Davis..... 444

Evaluation of Lamb Feeding Tests of Alfalfa Hay Grown on a Low-Sulfur Soil. V. V. Rendig and W. C. Weir..... 451

The Effect of Nitrogen Fertilization on the Nutritive Value of Orchardgrass Hay. B. R. Poulton, G. J. Macdonald and G. W. Vander Noot..... 462

The Effect of Phosphate Fertilization of Alfalfa on Growth, Reproduction, and Body Composition of Domestic Rabbits. W. W. Heinemann, M. E. Ensminger, W. E. Ham and J. E. Oldfield.. 467

The Digestibilities of Interior Live Oak and Chamise by Deer and Sheep. Harold D. Bissell and W. C. Weir..... 476

A Comparison of the Live Probe and Lean Meter for Predicting Various Carcass Measurements of Swine. A. M. Pearson, J. F. Price, J. A. Hoefer, L. J. Bratzler and W. T. Magee..... 481

Splitting and Cutting Errors in Swine Carcass Evaluation. E. L. Lasley and E. A. Kline..... 485

Bruised Tissue. II. Determination of the Age of a Bruise. M. K. Hamdy, L. E. Kunkle and F. E. Deatherage..... 490

Bruised Tissue. III. Some Factors Affecting Experimental Bruises. M. K. Hamdy, L. E. Kunkle, M. S. Rheins and F. E. Deatherage 496

Isolation and Identification of Compounds from Autolyzed Yeast, Alfalfa Meal, and Casein Hydrolysate with Celluloytic Factor Activity for Rumen Microorganisms *In Vitro*. B. A. Dehority, O. G. Bentley, R. R. Johnson and A. L. Moxon..... 502

Feedlot Bloat Studies. II. Physical Factors Involved in the Etiology of Frothy Bloat. D. R. Jacobson, I. L. Lindahl, J. J. McNeill, J. C. Shaw, R. N. Doetsch and R. E. Davis..... 515

The Carotene and Vitamin A Content of Plasma and Liver of Range Hereford Cows and Their Calves in the Northern Great Basin. R. R. Wheeler, P. H. Weswig, W. F. Brannon, F. E. Hubbert, Jr. and W. A. Sawyer..... 525

News and Notes..... 537

THE EFFECT OF STEROID HORMONES ON FATTENING LAMBS [1]

H. A. HENNEMAN, R. E. RUST AND J. MEITES [2, 3]
Michigan State University

SUBCUTANEOUS implantation of stilbestrol pellets has been shown to increase rate of gain and feed efficiency in feeder lambs with a consequent lowering of carcass grade (Andrews *et al.*, 1949, 1953; Jordan, 1950; Means *et al.*, 1953; O'Mary *et al.*, 1952; Pope *et al.*, 1950). Meites *et al.* (1952) observed an increase in finish and weight of heifers and cows implanted with combinations of stilbestrol and progesterone. Galloway *et al.* (1952) observed an increased rate of gain, feed efficiency and carcass grade in lambs using a combination implant of progesterone-stilbestrol.

The purpose of these experiments was to determine the effect of combination treatments of steroid hormones on lamb growth and fattening in an attempt to improve gain and feed efficiency without lowering the carcass grade and value.

Materials and Methods

Three experiments were conducted, the first in the fall of 1952 and the last in the fall of 1954. In every case the lambs were purchased and placed on feed before hormone treatments were begun. During the pre-implant period, weekly weights were taken and the daily gain and individual lamb weights were used in lotting the lambs for experiment.

The lambs were fed a ground mixture of alfalfa hay, yellow corn and 5% linseed oil meal. The mixture contained 70% hay (by weight) at the start and was reduced to 40% during the first 4 weeks of feeding. In the first two experiments the lambs were finished on the ration of 40% hay, 55% corn and 5% linseed oil meal. In experiment 3 the hay was reduced to 35% and the corn increased accordingly for the last month of feeding. Trace mineral salt and steamed bone meal were fed free-choice.

In all three experiments the following data were obtained: weekly weights of individual lambs, feed consumption by treatments, live grades, carcass grades and dressing percents. Pellet residues were recovered, cleaned of animal tissue, washed in distilled water, dried in vacuo and weighed on a Roller-Smith balance. Variations in experimental design, treatments and data collected are listed for each experiment.

The data were analyzed statistically by the analysis of variance and the "Student" t test.

[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 1962.
[2] Departments of Animal Husbandry and Physiology and Pharmacology, East Lansing.
[3] This work was supported by grants from Chemical Specialties Co., Inc., New York, New York.

Experiment I. Ninety Texas lambs averaging 80 lb. were divided into six treatments as follows: controls; 250 mg. progesterone; 250 mg. progesterone and 10 mg. estradiol; 10 mg. estradiol; 100 mg. delta 4 androstene 3, 17 dione (androgen); and 60 mg. testosterone. Ewes and wethers were included in each treatment to observe sex differences and sex treatment interactions.

All hormone pellets were implanted subcutaneously in the sub-maxillary region with a trocar and canula.

The lambs were all slaughtered when the lambs on one treatment averaged 100 lb.; therefore, the lambs were on feed only 28 days following implantation.

Mechanical separation of fat, lean and bone was made of the 9-rib rack. The percents fat, lean and bone in the carcass were calculated from the rack data according to the method of Hankins (1947). The percentage of moisture in the external fat was obtained for each lamb. From four composite samples per treatment (two each from the ewes and wethers) of fat and of the longissimus dorsi muscle; the percents moisture, ether extract and protein of both the fat and lean were determined.

Experiment II. Thirty-six lambs averaging 74 lb. were divided into treatments as follows: controls; 100 mg. progesterone and 10 mg. estradiol; 250 mg. progesterone and 10 mg. estradiol. One-half the lambs on each treatment were ewes and the other half were wethers.

All of the lambs were fed 42 days following hormone implantation and then slaughtered. The hormone pellets were implanted subcutaneously in the sub-maxillary region.

In addition to the data obtained similar to Experiment I, carcass shrinkage from hot to 48-hour cold carcass weight and histological sections of various visceral, endocrine and genital tissues were obtained.

Experiment III. Ninety-six Texas wether lambs averaging 81 lb. and 64 native Columbia and Corriedale sired lambs out of western ewes, averaging 70 lb., were divided equally into treatments as follows: controls; 100 mg. testosterone and 10 mg. estradiol; 50 mg. testosterone and 10 mg. estradiol; 10 mg. estradiol plus a second implant of 10 mg. estradiol 45 days later; 125 mg. progesterone and 5 mg. estradiol; 250 mg. progesterone and 10 mg. estradiol; 3 mg. stilbestrol daily in the feed; and 250 mg. progesterone and 10 mg. estradiol with 5% of the ration prime tallow as a replacement for corn. After three weeks on feed the amount of prime tallow was reduced to 2.5% of the ration.

The hormone pellets were implanted subcutaneously with a trocar and canula in the ear. Stilbestrol was pre-mixed with linseed oil meal before mixing with the entire ration. The prime tallow was heated and poured on the hay as it entered the mixer.

In an attempt to simulate standard marketing practice and because of the initial variation in liveweight, the lambs remained on feed until they reached

approximately 110 lb. liveweight or until they were fattened sufficiently for the choice grade at a lighter weight.

One or two lambs in each lot were making very unsatisfactory gains. With their distribution throughout all of the lots, it was felt their condition was not due to treatment and they were removed from the experiment after one month on feed.

Thirty-four of the ewe lambs were not slaughtered; therefore, live grades on these were used along with carcass grades on the other 112 lambs. Grades were assigned numerical values (low good, 1; average good, 2; high good, 3; etc.) to facilitate statistical analysis.

Results

Results of the three experiments are presented in tables 1, 2 and 3 for experiments I, II and III, respectively.

Growth Rate. In the first two experiments the implantation of combinations of progesterone and estradiol increased the daily gain of lambs 27 to 42% over the controls. Implants of the single hormones, progesterone, estradiol, delta 4 androstene 3, 17 dione and testosterone did not significantly increase daily gains over the control lambs.

In Experiment III all of the hormone treated lambs gained significantly faster than the control lambs. The highest gains were obtained with the combination implant of 50 mg. testosterone and 10 mg. estradiol (0.61 lb. per day), and these were significantly greater than gains of lambs orally fed 3 mg. stilbestrol daily; or an implant of 250 mg. progesterone and 10 mg. estradiol with inedible tallow added to the ration; or an implant of 125 mg. progesterone and 5 mg. estradiol. The average daily gain of lambs receiving 250 mg. progesterone and 10 mg. estradiol was 0.53 lb. and was not significantly less than the average gain of lambs implanted with 50 mg. testosterone and 10 mg. estradiol.

Feed Consumption. Feed data were obtained by treatments and statistical treatment was not possible. However, the data indicated an increased feed consumption by lambs receiving implants of progesterone and estradiol, testosterone and estradiol, testosterone, and orally fed stilbestrol as compared to the control lambs. By incorporating prime tallow in the ration a lower daily feed intake (3.05 lb.) resulted as compared to the control lambs (3.38 lb.). While the tallow had been stabilized, there was some question as to whether or not it had become rancid during storage. Therefore, further work would be necessary to draw any conclusions as to the advisability of adding inedible fats to the ration.

Feed Efficiency. In the first two experiments the lambs receiving combination implants of progesterone and estradiol required 17 to 32% less feed per 100 lb. gain than the control lambs. These were the only treatments in which a saving of feed was indicated when compared to the controls.

The performance of the control lambs in the third experiment (0.28 lb. average daily gain and 1209 lb. of feed per 100 lb. of gain) tends to make

the data for all of the treatments significant. However, the more efficient lambs of the hormone treatments were those receiving combination implants of 250 mg. progesterone and 10 mg. estradiol, with or without fat added to the ration. The lambs receiving 3 mg. of stilbestrol orally required the most feed per 100 lb. of gain of all of the hormone treatments.

TABLE 1. RESULTS OF EXPERIMENT I

Item	Control	250 mg. progest.	250 mg. progest. 10 mg. estradiol	10 mg. estradiol	100 mg. androgen	60 mg. Testost.
	1	2	3	4	5	6
No. of lambs	20	14	14	14	14	14
Av. initial wt., lb.	80.9	80.9	81.9	79.6	80.5	80.2
Av. final wt., lb.	95.5	92.7	100.4	91.7	93.4	96.2
Av. daily gain, lb.	0.52	0.42	0.66**	0.43	0.46	0.57
Dressing percent	46.3	46.7	46.5	49.2**	45.4	46.3
Av. carcass grade [d]	4.8	4.1	4.6	3.9	4.1	4.6
Feed per lb. of gain						
Alfalfa hay	3.30	3.98	2.82	3.97	3.80	3.27
Corn	3.78	4.57	3.21	4.52	4.31	3.75
Linseed oil meal	.37	.45	.32	.45	.43	.37
Total, lb.	7.45	9.00	6.35	8.94	8.54	7.39
Daily feed per lamb						
Alfalfa hay	1.72	1.69	1.86	1.71	1.75	1.87
Corn	1.97	1.93	2.12	1.95	1.98	2.14
Linseed oil meal	.19	.19	.21	.19	.19	.21
Total, lb.	3.88	3.81	4.19	3.85	3.92	4.22
Mechanical separation						
% Fat	25.0	25.9[b]	26.9[a]	22.9	24.5	24.6
% Lean	57.1	56.4	56.0	59.0	57.7	57.0
% Bone	16.9	16.7	16.2	17.1	17.3	17.4
Chemical analysis						
Moisture external fat, %	10.3	11.5	11.4	12.0	9.6	10.9
Fat						
% Protein	4.2	4.2	4.3	4.5	4.1	4.3
% Ether extract	81.3	80.2	79.4	79.6	82.1	80.1
% Moisture	14.2	15.4	16.2	16.0	13.7	15.5
Lean						
% Protein	21.5	21.2	22.2*	21.4	21.1	21.0
% Ether extract	5.1	5.4	5.5	5.3	4.5	5.4
% Moisture	72.2	72.0	71.6	72.5	73.3	72.2
Pellet absorption, %	—	46.8	57.9	90.0+[c]	90.0+[c]	62.1

** Significantly higher than controls ($P<.01$).
* Significantly higher than all other treatments ($P<.05$).
[a] Significantly higher than Lot 4 ($P<.01$); Lot 5 ($P<.05$).
[b] Significantly higher than Lot 4 ($P<.05$).
[c] Pellets absorbed or residues were impossible to separate from tissue and weigh.
[d] Numerical value 3, high good; 4, low choice, etc.

TABLE 2. RESULTS OF EXPERIMENT II

Item	Control 1	100 mg. progesterone 10 mg. estradiol 2	250 mg. progesterone 10 mg. estradiol 3
No. of lambs	11	11	11
Av. initial wt., lb.	74.8	74.1	75.1
Av. final wt., lb.	95.8	104.0	103.4
Av. daily gain, lb.	.50	.71**	.68**
Av. slaughter wt., lb.	90.3	98.0	97.5
Av. 36 hour shrink, lb.	5.5	6.0	5.9
Dressing percent	49.2	47.6**	47.7**
Percent carcass shrink (48 hr.)	3.2	3.3	3.5
Av. carcass grade	3.7	3.9	3.8
Feed per lb. of gain			
Alfalfa hay	3.96	2.99	3.07
Corn	5.22	3.93	4.02
Linseed oil meal	.48	.36	.37
Total, lb.	9.66	7.28	7.46
Daily feed per lamb			
Alfalfa hay	1.98	2.13	2.06
Corn	2.61	2.80	2.71
Linseed oil meal	.24	.26	.25
Total, lb.	4.83	5.19	5.02
Mechanical separation			
% Fat	20.08	20.64	19.51
% Lean	60.26	59.50	60.99
% Bone	19.66	19.86	19.50
Chemical analysis			
% Moisture external fat	9.6	10.6	11.8**
Fat			
% Protein	5.0	4.7	4.2
% Ether extract	78.6	77.7	74.8
% Moisture	16.1	17.0	19.5*
Lean			
% Protein	21.0	21.8	21.3
% Ether extract	4.4	3.6	4.2
% Moisture	73.8	74.3	73.7
Pellet absorption, %	....	46.5	....

** Significantly different from the controls ($P<.01$).
* Significantly different from Lots 1 and 2 ($P<.05$).

Lamb Behavior and Feedlot Losses. Lambs receiving implants of androgens, estradiol or testosterone and estradiol in combination exhibited the most undesirable behavior in the feedlot. There was a great deal of

riding, fighting and restlessness especially during the first two weeks after implant. The control lambs were the most docile; however, the lambs receiving combination progesterone and estradiol implants or oral stilbestrol were not objectionable in their behavior. Mammary development was observed in the lambs receiving hormone treatment.

TABLE 3. RESULTS OF EXPERIMENT III

Item	Control 1	100 mg. testosterone 10 mg. estradiol 2	50 mg. testosterone 10 mg. estradiol 3	10 mg. estradiol plus second implant of 10 mg. in 6 wks. 4	125 mg. prog. 5 mg. estradiol 5	250 mg. prog. 10 mg. estradiol 6	Fed 3 mg. stilbestrol daily 7	250 mg. prog. 10 mg. estradiol plus fat 8
No. of lambs	18	18	19	18	18	19	17	19
Av. initial wt., lb.	77.6	76.5	76.0	77.8	77.5	76.5	78.3	76.3
Av. final wt., lb.	95.0	104.3	103.9	103.7	103.2	103.6	100.8	102.7
Av. daily gain, lb.	.28**	.52	.61[a]	.52	.51	.53	.45	.47
Av. No. days on feed	62	53	46	49	51	51	50	56
Dressing percent	47.6[b]	46.7	45.8	45.8	45.4	45.3	46.4	45.3
Av. carcass grade	3.7[c]	2.6	2.3	2.6	2.6	2.8	3.0[d]	2.5
Av. selling price per Cwt. ($)	19.43	18.10	17.89	18.27	18.13	17.77	18.28	17.92
Feed per lb. of gain								
Alfalfa hay	4.56	2.94	2.86	3.16	3.08	2.75	3.44	2.51
Corn	6.93	4.46	4.29	4.81	4.61	4.16	5.01	3.45
Linseed oil meal	.60	.39	.38	.42	.40	.36	.44	.33
Prime tallow	—	—	—	—	—	—	—	.21
Total, lb.	12.09	7.79	7.53	8.39	8.09	7.27	8.89	6.50
Feed cost per 100 lb. gain, ($)	25.83	16.65	16.07	17.97	17.23	15.52	18.85	14.55
Feeding margin ($)	—6.40	1.45	1.82	0.30	0.90	2.25	—0.57	3.37
Daily feed per lamb								
Alfalfa hay	1.27	1.54	1.75	1.66	1.56	1.47	1.55	1.18
Corn	1.94	2.33	2.63	2.52	2.34	2.22	2.26	1.62
Linseed oil meal	.17	.20	.23	.22	.20	.19	.20	.15
Prime tallow	—	—	—	—	—	—	—	.10
Total, lb.	3.38	4.07	4.61	4.40	4.10	3.88	4.01	3.05
Pellet absorption, %								
29-day implants	—	74.2(2)	51.9(4)	91.6(4)	18.4(5)	22.7(3)	—	—
50-day implants	—	89.2(9)	81.6(7)	78.9(8)	38.8(5)	37.4(10)	—	34.3(13)

** Lot 1 significantly less gain than all other Lots ($P<.01$).
[a] Lot 3 significantly more gain than Lots 7 & 8 ($P<.01$) and Lot 5 ($P<.05$).
[b] Lot 1 significantly higher dressing % than Lots 5, 6 & 8 ($P<.01$) and Lots 3 & 4 ($P<.05$).
[c] Lot 1 significantly higher carcass grade than Lots 2, 3, 4, 5, 6 & 8 ($P<.01$) and Lot 7 ($P<.05$).
[d] Lot 7 significantly higher carcass grade than Lot 3 ($P<.05$).
( ) Number of lambs from which pellets were recovered.

No deaths occurred in the first experiment. In Experiment II one wether lamb that was implanted with 100 mg. progesterone and 10 mg. estradiol exhibited an inability to urinate the last three days of the experiment. On examination, calculus was observed at the end of the urethra. One wether lamb in Experiment III died 25 days after being implanted with 10 mg. of estradiol. On autopsy, the urethra had ruptured anterior to the prostate region and 1000 ml. of urine was present in the abdominal cavity. Also in

the last experiment, one ewe lamb receiving an implant of 125 mg. progesterone and 5 mg. estradiol was slaughtered due to a prolapsed uterus.

Marketing Shrink. The lambs on Experiment II were slaughtered at the University meats laboratory and it was possible to obtain both a final feedlot weight and a slaughter weight after the lambs were off feed 36 hours. There was no significant difference in shrinkage due to treatment.

Dressing percentage. In Experiment I, dressing percents were calculated on the basis of final feedlot weight and cold carcass weight with 24-hour shrink. Only the lambs implanted with 10 mg. estradiol dressed significantly higher than the control lambs. There was no difference in dress between the controls and any other hormone treatment.

In Experiment II, the dress was based on a slaughter weight after 36 hours off feed and a 48-hour cold carcass weight. In this experiment the control lambs dressed significantly higher than lambs implanted with combinations of progesterone and estradiol.

When lambs were topped out of the feedlot at about 110 lb. in weight or when they had reached a choice finish, the control lambs dressed higher than lambs implanted with 50 mg. testosterone and 10 mg. estradiol, estradiol, or combinations of progesterone and estradiol. Lambs being fed 3 mg. stilbestrol or implants of 100 mg. testosterone and 10 mg. estradiol did not differ significantly in dressing percent compared to the controls. These dressing percents were calculated on a final feedlot weight and hot carcass weight.

Carcass Shrink. Only in Experiment II was it possible to obtain carcass shrink from hot to 48-hour cold carcass weight. In this case no significant difference in carcass shrinkage was observed.

Carcass Grade. All the lambs were slaughtered in Experiment I, 28 days after the treated lambs were implanted and in Experiment II all lambs were slaughtered 42 days after implant. In both of these experiments there was no significant difference in carcass grade.

However, in the third experiment when lambs were topped out of the feedlot, the control lambs graded significantly higher than all of the hormone treated lambs.

Mechanical Separation. The percents fat, lean and bone of the carcass were estimated from the separation of the nine-rib rack in the first two experiments. No significant differences were observed in percent fat between the control lambs and hormone treated lambs. In Experiment I the lambs implanted with the combination of 250 mg. progesterone and 10 mg. estradiol had a higher percentage of separable fat than lambs implanted with estradiol or synthetic androgen. Also the progesterone implanted lambs had a higher percentage of separable fat than estradiol implanted lambs. In Experiment II an implant of 100 mg. progesterone and 10 mg. estradiol yielded more fat than implants of 250 mg. progesterone and 10 mg. estradiol.

The percentages of lean and bone vary inversely with percent fat and

therefore the analysis of covariance was used to study differences in percents lean and bone. There was no significant difference between the control and treated lambs in either Experiment I or II.

Chemical Analysis.[4] In Experiment I there was no significant difference in percent moisture in the external fat. However, in Experiment II the external fat of lambs implanted with 250 mg. progesterone and 10 mg. estradiol was significantly higher in moisture than the control lambs.

When samples of both external and intermuscular fat were mixed and analyzed for percents moisture, protein and ether extract, higher values for moisture were obtained than when only external fat was analyzed. This may be due to a higher moisture content of intermuscular fat but it is suspected that more connective tissue was included in the mixed sample. No significant difference between control and treated lambs were observed in percents moisture, ether extract or protein in Experiment I. In Experiment II the percent moisture was again higher in lambs implanted with 250 mg. progesterone and 10 mg. estradiol. However, there were no differences in percents ether extract and protein.

From a chemical analysis of a sample of the longissimus dorsi for percents protein, ether extract and moisture in the first two experiments only one significant difference was found. Lambs receiving the combination implant in the first experiment of progesterone and estradiol had a higher percentage of protein than all other lots of lambs. This difference was not observed in the second experiment.

Pellet Absorption. Absorption of the combination progesterone, estradiol pellets was more efficient in Experiments I and II when the pellets were implanted in the submaxillary region as compared to Experiment III when the pellets were implanted in the ear.

Pellets of estradiol, delta 4 and androstene 3, 17 dione and combinations of testosterone-estradiol were more rapidly absorbed than progesterone or combination progesterone-estradiol pellets.

Histological Examination.[5] Both the ewe and wether lambs in Experiment II receiving progesterone-estradiol implants exhibited enlarged Hassel's corpuscles of the thymus. The thyroid glands of the ewe and wether lambs implanted with 250 mg. progesterone and 10 mg. estradiol showed an increased amount of colloid. The accessory sex glands of the wethers implanted with progesterone-estradiol pellets exhibited hyperplasia, cystic dilation and keratinization of the epithelial structures. No abnormalities were observed in the genital organs of the ewe lambs.

Feeding Margin. In Experiment III the lambs were sold on a carcass grade and weight basis and the value of the lambs by treatments could be calculated. Lambs implanted with 250 mg. progesterone and 10 mg. estradiol with or without fat added to the ration and lambs implanted with 50 mg. testosterone and 10 mg. estradiol sold for the least value per hundred

[4] Chemical determinations by E. J. Benne, Agricultural Chemistry Department.
[5] Histology by F. Thorpe, Jr., Animal Pathology Department.

pounds due to their lower dressing percent and carcass grade. However, their feed efficiency was such that the widest profitable feeding margins were obtained with these lambs.

The control lambs and lambs fed stilbestrol were the higher dressing, higher grading lambs selling for the highest price but their high feed requirement resulted in a negative feeding margin.

Sex. Data were obtained by sex in the first two experiments and no sex-treatment interaction was observed. In Experiment I the ewe lambs graded significantly higher (average choice) as compared to wethers (low choice). The ewe lambs had a higher percentage of separable fat (25.8% as compared to 24.1% for wethers). In Experiment II the wethers yielded 20.7% separable fat and the ewe lambs 19.5%. These were the only significant differences between the sexes.

Type of Lamb. In Experiment III an analysis was made according to the type of lamb. Texas lambs gained significantly faster (0.56 lb.) than native Corriedale and Columbia sired lambs out of western ewes (0.44 lb.). The native lambs graded one-third of a grade higher than Texas lambs which was statistically significant. No other differences were observed.

Discussion

Lambs implanted with a combination of 250 mg. progesterone and 10 mg. estradiol gained more rapidly, with a lower feed requirement, than control lambs. When the lambs in all treatments were fed the same length of time, the combination implant of progesterone-estradiol did not lower the carcass grade. However, when lambs were topped out for slaughter at either 110 lb. final feedlot weight or when the lambs were fat enough to grade choice at a lighter weight, the implanted progesterone-estradiol lambs graded significantly lower in the carcass than control lambs. Pope *et al.* (1950) and O'Mary *et al.* (1952) implanted a combination of 60 mg. progesterone and 12 mg. stilbestrol and reported increased gains and feeding efficiency with a lower carcass grade and dressing percentage.

The amounts and ratios of progesterone and estradiol were varied with similar results. The combination of testosterone and estradiol also produced similar results.

Bell *et al.* (1953) observed high mortality due to urinary calculi and prolapse of the rectum in lambs receiving a second stilbestrol implant 70 days after the first implant. On macroscopic examination at slaughter in these experiments all of the hormone treatments caused hypertrophy of the secondary sex organs in wether lambs. However, the only losses experienced in these trials were as follows: one wether lamb receiving an implant of 100 mg. progesterone and 10 mg. estradiol exhibited urinary calculi; one wether lamb implanted with 10 mg. estradiol died with a ruptured urethra; and one ewe lamb implanted with 125 mg. progesterone and 5 mg. estradiol was slaughtered due to a prolapsed uterus.

Hormone treated lambs also exhibited signs at slaughter usually asso-

ciated with age, such as difficulty in pelting and in obtaining the break-joint.

The absorption efficiency of progesterone implants or combination implants containing progesterone is low in comparison to other hormones and in this experiment was affected by the site of implantation; the ear being less efficient than the sub-maxillary region. However, the ear would would be much less objectionable in commercial practice due to the pellet residues.

The high feed requirement and low daily gain of the control lambs in Experiment III make the data for the hormone treated lambs look impressive. Of the hormone treatments, the feeding of 3 mg. of stilbestrol daily produced the slower gaining lambs with the higher feed requirement. While the stilbestrol fed lambs tended to grade higher than implanted lambs, they still graded significantly lower than control lambs.

Summary

Three experiments were conducted with fattening lambs to determine the effect of various steroid hormones and combinations of hormones on feedlot performance and carcass quality.

Lambs implanted with a combination of 250 mg. progesterone and 10 mg. estradiol exhibit more rapid, economical gains and a larger daily feed intake than control lambs. When all lambs are slaughtered after the same length of time on feed, there is no significant difference in carcass grade between controls and progesterone-estradiol implants. However, when lambs are topped out of the feedlot at various intervals, the progesterone-estradiol implanted lambs have a lower dressing percent and carcass grade than lambs without hormone treatment. The progesterone-estradiol implant tended to produce fat with a higher moisture content.

A combination implant of 100 mg. progesterone and 10 mg. estradiol compared favorably with the 250 mg. progesterone-10 mg. estradiol implant but one lamb was afflicted with urinary calculi.

A combination implant of 50 mg. testosterone and 10 mg. estradiol produced similar results as progesterone-estradiol with the exception of more restlessness in the feedlot.

The feeding of 3 mg. stilbestrol daily produced no significant improvement over the progesterone-estradiol implants, although the lambs tended toward an improved dressing percent with a slower daily gain and higher feed requirement.

Hormone treated wether lambs exhibit hypertrophy of the secondary sex organs and both wethers and ewes exhibit mammary development. Difficulty in pelting and obtaining the break joint was experienced at slaughter.

The absorption of progesterone is low in comparison to other hormones and is affected by the site of implantation; the ear being less efficient than the sub-maxillary region. The ear was a satisfactory implantation site for hormones other than progesterone.

Implants of 250 mg. progesterone; 100 mg. delta 4 androstene 3, 17 dione; 10 mg. estradiol; or 60 mg. testosterone produced no advantages over control lambs.

Literature Cited

Andrews, F. N. and W. M. Beeson. 1953. The effects of various methods of estrogen administration on the growth and fattening of wether lambs. J. Animal Sci. 12:182.

Andrews, F. N., W. M. Beeson and Claude Harper. 1949. The effect of stilbestrol and testosterone on the growth and fattening of lambs. J. Animal Sci. 8:518.

Bell, T. Donald, Walter H. Smith and A. B. Erhart. 1953. Use of stilbestrol in fattening lambs. Kan. Agr. Exp. Sta. Circ. 297.

Galloway, J. H., L. J. Bratzler, L. H. Blakeslee and J. Meites. 1952. Effects of stilbestrol-progesterone implants on the growth and carcass quality of lambs. Mich. Agr. Exp. Sta. Quart. Bul. 35:68.

Hankins, O. G. 1947. Estimation of the composition of lamb carcasses and cuts. U.S.D.A. Tech. Bul. 944.

Jordan, R. M. 1950. The effect of stilbestrol on fattening lambs. J. Animal Sci. 9:383.

Means, T. M., F. N. Andrews and W. M. Beeson. 1953. The effects of hormones on the growth and fattening of lambs. J. Animal Sci. 12:176.

Meites, J., E. P. Reineke and C. F. Cairy. 1952. Induction of lactation in dairy cattle by diethylstilbestrol-progesterone implants. J. Dairy Sci. 25:504.

O'Mary, C. C., A. L. Pope, G. D. Wilson, R. W. Bray and L. E. Casida. 1952. The effects of diethylstilbestrol, testosterone, and progesterone on growth and fattening and certain carcass characteristics of western lambs. J. Animal Sci. 11:656.

Pope, A. L., C. C. O'Mary, W. E. Batterman, R. W. Bray and L. E. Casida. 1950. The effect of certain steroid hormones on the growth, fattening and carcass quality of heavy lambs. J. Animal Sci. 9:680.

GROWTH AND FATTENING STIMULATION IN EWE LAMBS BY CERTAIN ANDROGENIC AND ESTROGENIC COMPOUNDS [1]

BRUCE TAYLOR, W. H. HALE AND WISE BURROUGHS [2]

Iowa Agricultural Experiment Station, Ames

SINCE 1949 it has been demonstrated repeatedly that diethylstilbestrol (stilbestrol) administered subcutaneously as a pellet implant will significantly improve rate of gain and efficiency of feed utilization of feeder lambs (Andrews *et al.*, 1949; Jordan, 1950, 1953; Pope *et al.*, 1950; Klosterman *et al.*, 1951; Galloway *et al.*, 1952; O'Mary *et al.*, 1952; Means *et al.*, 1953; Clegg and Cole, 1954; Bell *et al.*, 1954; Richard and Dinusson, 1954; Clegg *et al.*, 1955; Andrews *et al.*, 1956). This stimulation in rate of gain and feedlot efficiency has often been accompanied by a lowering of carcass grade. In a majority of these trials the sex of the feeder lambs was not reported.

Recently, the oral use of stilbestrol in lambs has shown some promise in stimulating gains and in improving efficiency of feed utilization without materially lowering carcass grade or producing undesirable side effects (Hale *et al.*, 1953, 1955; Jordan, 1956; Light *et al.*, 1956). Progesterone combined with estradiol and administered as a pellet implant has also been shown to increase live weight gains and feed efficiency in lambs but usually at the expense of carcass grade (Galloway *et al.*, 1952; Acker *et al.*, 1955, Smith *et al.*, 1955; Andrews *et al.* 1956; Bell and Erhart, 1956). Stimulation in rate of gain by injections of testosterone has been reported by Burris *et al.* (1954) for steers and heifers and by O'Mary *et al.* (1952) and Means *et al.* (1953), using testosterone propionate implants, with lambs. Others (Pope *et al.*, 1950; Johnson *et al.*, 1956; Andrews *et al.*, 1956) reported that testosterone propionate had no effect on rate of gain in feeder lambs.

The purpose of this study was to determine if androgens or estrogens, used singly or in combination, would produce beneficial effects on growth rate and efficiency of feed utilization in ewe lambs without producing undesirable side effects or adverse effects on carcass quality.

Materials and Methods

This study was composed of three individual feeding and two group feeding experiments involving 324 Western ewe lambs. In the individual feeding experiments either four or six lambs were allotted at random to different rations from body weight outcome groups. The individually-fed

[1] The authors express acknowledgment to Ciba Pharmaceutical Products, Inc. for the methyl testosterone and testosterone propionate used.

[2] Journal paper No. J-3060 of the Iowa Agricultural Experiment Station, Ames, Project No. 869.

lambs received mixed rations and were allowed to feed from individual self feeders for two 3-hour periods each day. The starting ration consisted of ground alfalfa hay, 50%; cracked corn, 34.5%; cane molasses, 10%; and soybean oil meal, 5.5%. Corn was increased, replacing hay, so that the mixture comprised only 35 to 40% hay by the 28th day of each trial. The estrogen or androgen additions were mixed with soybean oil meal in such amounts that the desired level would be consumed if the lambs averaged 3.5 lb. feed intake of the complete ration per day. Experiment 1 was conducted from December to March, 1953–54; with white-faced lambs averaging 72 lb. initially; Experiment 2, March to June, 1954 with black-faced lambs averaging 79 lb. initially; and Experiment 3, October to January 1954–55 with 74-lb. black-faced lambs. All individually-fed lambs were weighed each 14th day.

In Experiment 4, a group feeding trial, 100 white-faced ewe lambs averaging 71 lb. initially were assigned from body weight-outcome groups to 10 lots of 10 lambs each. Two lots were fed together in testing five experimental feeding treatments. One of the two lots on each treatment was randomally selected and shorn two days before the start of the trial. The remaining lambs were left in fleece estimated to grade number two pelts. In Experiment 5, 120 white-faced ewe lambs averaging 78 lb. initially were assigned from body weight-outcome groups to six lots of 20 lambs each. Two lots were randomly assigned to each of three experimental treatments. The group-fed lambs were fed twice daily on cracked corn (Experiment 4) or shelled corn (Experiment 5), and alfalfa hay ad libitum; and .25 lb. of 44% soybean oil meal. After 28 days of feeding the alfalfa hay was limited to 1.5 lb. per lamb per day and all refused hay weighed back before the next feeding. The stilbestrol additions were made in the soybean oil meal. The lambs were weighed individually at 14-day intervals in Experiment 4 and at 21-day intervals in Experiment 5.

A vasectomized yearling ram was used regularly, in Experiment 4, to determine if any treatment effect would result in the ewe lambs accepting the ram.

Results

Weight Gains and Feed Consumption. The data for live weight gains and feed consumption are presented in tables 1 and 2 for the individually-fed and group-fed lambs, respectively. The shorn and wooled sub-groups of Experiment 4 are presented as one lot, since the effect of wool was not significant. In Experiment 1, testosterone propionate injected at the rate of 3.3 mg. per 100 lb. of body weight per day increased live weight gains by 38% over the control lambs and this difference was statistically significant. Methyl testosterone, in the same experiment, fed at levels of either 8.5 or 42.9 mg. per lamb daily increased gains by approximately 16% and this was significantly greater than the gains made by the control lambs. How-

TABLE 1. WEIGHT GAINS AND FEED CONSUMPTION IN INDIVIDUALLY FED EWE LAMBS WITH CERTAIN ANDROGENIC AND ESTROGENIC COMPOUNDS

Exp. No.	Lambs per group	Days fed	Treatment								
			Control	Methyl[b] test. orally	Methyl[c] test. orally	Test. prop. injection	Test. prop. orally	DES orally	Test. prop. and DES	Methyl[a] test. and DES	Methyl[d] test. then DES
			Average daily gain, lb.								
1*	4	84	.38	.44	.44	.51					
2	4	82	.32		.38	.38	.36	.36	.42	.36	.36
3	6	84	.44		.39	.35		.40	.40	.43	
			Average daily feed consumption, lb.								
1			3.22	3.51	3.53	3.61					
2			3.36		3.62	3.55	3.47	3.48	3.65	3.62	3.49
3			3.70		3.27	3.31		3.55	3.35	3.61	

* Treatment effects significant at P=.05 or less. L.S.D.=0.057.

[a] Methyl testosterone and DES. 47 mg. methyl testosterone per lamb per day plus 2 mcg. stilbestrol per pound of feed in Exp. 2, and 39.24 mg. methyl testosterone plus 2 mg. stilbestrol in Exp. 3.

[b] Methyl testosterone fed at the rate of 8.52 mg. per lamb daily.

[c] Methyl testosterone fed at the following rates per lamb daily: Exp. 1, 42.86 mg.; Exp. 2, 47 mg.; and Exp. 3, 29.24 mg.

Testosterone Propionate injection. Testosterone propionate partially dissolved in sesame oil and injected each 14th day at the rate of 3.3 mg. per 100 lb. body weight per day.

Testosterone Propionate orally. Fed at the rate of 60 mg. per lamb per day.

DES orally. Diethylstilbestrol fed at the rate of 2 mcg. per lamb per day in Exp. 2, and 2 mg. per lamb daily in Exp. 3.

Testosterone propionate and DES. injection as above plus 2 mcg. stilbestrol per pound of feed in Exp. 2, and 2 mg. of stilbestrol per lamb daily in Exp. 3.

[d] Methyl testosterone then DES. 47 mg. methyl testosterone per lamb daily for 35 days, then 2 mcg. stilbestrol per pound of feed for 47 days.

ever, in repeating these treatments, in Experiments 2 and 3, no such stimulation in rate of gain was observed. Neither did the feeding of testosterone propionate at approximately 20 times the injected level used in Experiment 2 nor the implanting of 30 mg. of the same drug in Experiment 4 have any effect on live weight gain. Feed intake was not affected significantly by methyl testosterone or testosterone propionate; however, in Experiment 6, the lambs implanted with testosterone propionate appeared to be easier to keep on feed than other lambs in the experiment.

Ewe lambs implanted with 250 mg. of progesterone and 10 mg. of estradiol, in Experiment 4, made 14.6% more gain than the control lambs (P=.01) and produced 100 lb. of gain on 110 lb. less feed.

In Experiment 5, the feeding of 3.5 mg. of stilbestrol per lamb daily increased gains significantly (19%) and reduced the amount of feed required per unit of gain without significantly altering feed intake as compared to control lambs. However, in Experiment 4, the feeding of either 1.4 or 2.67 mg. of stilbestrol daily significantly reduced live weight gains of ewe lambs. Feed intake was reduced, although not significantly, in this experiment and the lambs were very difficult to keep on a full feed of corn. Neither stilbestrol alone or stilbestrol combined with either methyl testosterone or testosterone propionate had a significant effect on rate of gain or feed consumption in the trials in which the lambs were individually fed.

TABLE 2. WEIGHT GAINS, FEED CONSUMPTION AND ANALYSES OF GROUP-FED LAMBS WITH CERTAIN HORMONE OR HORMONE-LIKE COMPOUNDS

		Experiment 4					Experiment 5		
Description	Rep.	Control	1.4 mg. DES	2.67 mg. DES	250 mg. prog. 10 mg. estradiol implant	30 mg. test. prop. implant	Control	2 mg. DES	3.5 mg. DES
No. of lambs	1	19	20	18	20	20	20	20	20
	2	—	—	—	—	—	19	20	20
Av. daily gain, lb.	1	.48	.42	.42	.55	.46	.36	.41	.44
	2	—	—	—	—	—	.36	.38	.42
Av. daily feed consumption, lb.	1	3.25	3.10	3.00	3.25	3.18	2.75	2.86	2.91
	2	—	—	—	—	—	3.07	3.05	2.95

Analysis of variance of average daily gains

Experiment 4				Experiment 5			
Source of variation	d.f.	M.S.	F	Source of variation	d.f.	M.S.	F
Reps. within wool group	17	.0305		Replicates	1	.00700	
Wool	1	.0286		Rations	2	.05435	21.31*
Rations	4	.0605	14.30**	Experimental error	2	.00255	
Rations vs. wool interaction	4	.0115		Lambs/pens	113	.00509	
Reps. vs. treatments	68	.00423					

* Treatment effect significant at P=.05 or less. L.S.D.=.036.
** Treatment effect significant at P=.01 or less. L.S.D.=.055.

TABLE 3. SHRINK IN CHILLING, DRESSING PERCENTAGE AND CARCASS GRADE IN INDIVIDUALLY FED EWE LAMBS WITH CERTAIN ANDROGENIC AND ESTROGENIC COMPOUNDS

Experiment No.	Lambs per group	Days fed	Treatment [a]								
			Control	Methyl test. orally	Methyl test. orally	Test. prop. injection	DES orally	Test. prop. and DES	Methyl test. and DES	Methyl test. then DES	Test. prop. orally
			Percent shrink in chilling								
1	4	84	2.97	1.64	1.63	3.29					
2	4	82	1.22		1.35	1.16	1.13	1.70	0.90	1.56	0.63
3	6	84	2.40		2.26	1.98	2.68	2.31	2.75		
			Dressing percentage, chilled basis								
1*			49.4	49.15	50.9	47.7					
2			44.4		45.5	43.6	45.7	45.0	45.3	45.5	45.5
3			50.6		50.1	49.4	50.0	50.0	48.7		
			Carcass grades [b]								
1**			7.25	7.00	7.75	4.50					
2			4.75		6.25	5.40	6.25	6.25	6.50	6.75	6.00
3			9.20		8.80	8.00	8.80	8.70	8.50		

* Treatment effect significant at P=.05 or less. L.S.D.=1.00.
** Treatment effect significant at P=.01 or less. L.S.D.=2.06.
[a] Explained in table 1.
[b] Carcass grades computed using the following numerical system: prime, 11; prime minus, 10; choice plus, 9; choice, 8; choice minus, 7; good plus, 6; good, 5; good minus, 4; utility plus, 3; and utility, 2.

Carcass Data. The data for shrink in chilling, government grades and dressing percentage are shown in tables 3 and 4 for the individually-fed and group-fed lambs, respectively. In Experiment 1, in which both testosterone propionate and methyl testosterone significantly improved rates of gain, the testosterone propionate produced growthy carcasses which lacked finish, graded a full grade lower than the control carcasses, showed the greatest loss in chilling, and yielded significantly less than did the control lambs. In subsequent trials, in which testosterone propionate had no effect on rate of gain, it had no observed effect on carcass quality. Methyl testosterone, in Experiment 1, improved carcass grade and fatness when fed at the rate of 42.9 mg. per lamb daily, but had no effect on carcass grade when fed at

TABLE 4. SHRINK IN CHILLING, DRESSING PERCENTAGE AND CARCASS GRADE IN GROUP-FED EWE LAMBS WITH CERTAIN HORMONE OR HORMONE-LIKE COMPOUNDS

		Experiment 4					Experiment 5		
Description	Rep.	Control	1.4 mg. DES orally	2.67 mg. DES orally	250 mg. prog. 10 mg. estradiol implant	30 mg. test. prop. implant	Control	2 mg. DES	3.5 mg. DES
Percent shrink in chilling	1	2.89	2.76	2.84	3.02	2.49	2.37	2.57	2.75
	2						2.89	2.60	2.75
Dressing percent	1	47.64	47.74	48.35	47.47	47.77	49.01	49.42	49.05
	2						51.26	50.05	49.98
Carcass grades [a]	1	5.89	5.65	5.94	4.80*	6.00	6.20	6.35	6.35
	2						5.50	5.15	5.05
Percent yearling carcasses	1	0.00	30.00	30.00	50.00	5.00	20.00	90.00	100.00
	2						26.30	80.00	95.00

* Treatment effect significant at P=.05 or less.
[a] Explained in table 2.

the rate of 8.5 mg. per lamb daily. In Experiments 2 and 3 methyl testosterone had no effect on carcass grade.

The implanting of 250 mg. of progesterone and 10 mg. of estradiol, in Experiment 4, resulted in 50% of the implanted animals yielding yearling carcasses; whereas the untreated control lambs yielded 100% lamb carcasses. Grading all carcasses on the lamb basis revealed a significant lowering of carcass grade due to implantation. Contributing to this lower grade were sunken loins, uneven finish and whiteness of bone. In addition the progesterone-estradiol-treated lambs proved extremely difficult to pelt.

The feeding of stilbestrol, at the levels used in the individual trials, (2 mg. per day or less) had no significant effect on rate of gain or carcass grades. However, each level of stilbestrol fed in this series of experiments made the pelts difficult to remove.

The feeding of stilbestrol, in the group feeding trials, had no significant effect on shrink in chilling, dressing percentage or on carcass grade where

TABLE 5. EFFECT OF CERTAIN ANDROGENIC AND ESTROGENIC COMPOUNDS ON THE UTERI AND OVARIES OF INDIVIDUALLY FED EWE LAMBS

Experiment No.	Lambs per group	Days fed	Treatment [a]								
			Control	Methyl test. orally	Methyl test. orally	Test. prop. injection	DES orally	Test. prop. and DES	Methyl test. and DES	Methyl test. then DES	Test. prop. orally
			Weight of uteri, grams								
1	4	84	18.61	15.76	17.23	17.06					
2	4	82	22.26		13.58	23.54	18.21	14.62	22.33	22.45	23.23
3**	6	84	24.04		18.57	29.18	24.34	22.01	15.78		
			Weight of ovaries, grams								
1			1.17	1.26	1.42	0.99					
2			0.89		1.04	0.94	0.74	0.70	0.90	0.85	1.07
3			1.22		1.07	1.16	1.04	0.99	0.68		
			Follicles of 2 mm. or larger, number								
3			1.83		1.20	1.16	0.00	0.00	0.00		

** Treatment effect significant at P=.01 less; L.S.D.=4.51.
[a] Explained in table 1.

all carcasses were graded on the lamb basis. However, the feeding of stilbestrol increased the percentage of carcasses classed as yearlings from none to 30% in Experiment 4, and from 30 to 85 and 98%, in Experiment 5, for the 2 mg. and 3.5 mg. levels, respectively.

The data for the observations on the uteri and ovaries are presented in tables 5 and 6 for the individually-fed and group-fed lambs, respectively. Methyl testosterone fed at levels of from 8.5 to 47 mg. per lamb daily or testosterone propionate whether injected, fed, or implanted had no significant effect on the size of the uteri, ovaries or on the number of developing follicles.

TABLE 6. EFFECT OF CERTAIN HORMONE OR HORMONE-LIKE COMPOUNDS ON THE UTERI AND OVARIES OF GROUP-FED EWE LAMBS

		Experiment 4					Experiment 5		
Description	Rep.	Control	1.4 mg. DES	2.67 mg. DES	250 mg. prog. 10 mg. estradiol	30 mg. test. prop. implant	Control	2 mg. DES	3.5 mg. DES
Wt. of uteri, gm.	1	27.74	29.58	35.65	45.47*	27.67	27.0	25.7	28.9
	2						28.3	31.8	31.6
Wt. of ovaries, gm.	1	1.11	1.03	0.70**	1.10	1.42	1.29	0.91	0.92
	2						1.37	1.11	0.88*
Number of follicles [a]	1	1.22	0.15	0.00	0.00	1.84	1.55	0.00	0.00
	2						0.95	0.15	0.00

* Treatment effect significant at P=.05 or less; L.S.D.=4.45 for wt. of uteri; 0.094 for wt. of ovaries.

** Treatment effect significant at P=.01 or less; L.S.D.=0.28.

[a] Only follicles of 2 mm. or more in diameter counted.

The implanting of 250 mg. of progesterone and 10 mg. of estradiol significantly increased the size of the uteri, reduced the number of developing follicles of 2 mm. size or larger to zero, but had no effect on the weight of the ovaries.

Levels of stilbestrol of 2.6 or 3.5 mg. per lamb daily tended to increase uterine size, and in Experiment 4 this effect was statistically significant. In both experiments the feeding of stilbestrol at these levels reduced the size of the ovaries and retarded follicle development. Lower levels of stilbestrol produced similar effects, but to a lesser degree.

The Liver, Thyroid and Adrenals. Weights of the liver, thyroid and adrenals were taken, for Experiment 1, in which both methyl testosterone and testosterone propionate produced a significant response in weight gains. Both of the androgens increased liver size and tended to reduce the size of the adrenals. Testosterone propionate significantly increased (P=.05) the weight of the thyroid, (41%) but histological examination showed no alteration from that observed in the control lambs. Methyl testosterone, on the other hand, significantly reduced the weight of the thyroid (28%) when fed at the rate of 8.5 mg. per lamb daily.

The Effect of Shearing. The effects of shearing (Experiment 4) are not presented in table form. Shorn lambs gained faster than their wooled pen mates on all treatments in the experiment, but correcting these gains for the extra shrink (above the wool clip) of shearing erased the advantage of shearing for all except the group implanted with testosterone propionate. In this case, the shorn lambs gained 27% faster than their wooled mates, but failed to gain significantly faster than the shorn lambs of the control group.

Other Observations. Excessive riding was evident among the ewe lambs by the fourth day after implantation with progesterone and estradiol. The first evidence of riding occurred on March 4 and on March 9 the vasectomized ram served the first lamb. This lamb accepted the ram again on March 10. Another lamb was served on March 14, 16, and 21, and on April 23. In all, 20% of the lambs in this group accepted the ram one or more times in the March 9 to April 23 period. The lambs in the control group as well as those in the two groups being fed stilbestrol would not accept the ram. One lamb in the group implanted with testosterone propionate did accept the ram, but no evidence of riding was observed in this lot.

It was observed that mammary development was stimulated by stilbestrol and by the progesterone-estradiol implant, but no attempt was made to measure this stimulation.

Nausea, with vomiting, and intestinal distress as evidenced by alternate lying down and standing were exhibited by some of the lambs in the two lots of experiment 4 which were fed stilbestrol. These two groups were started on levels of 2 and 3.5 mg. of stilbestrol, respectively, but because of difficulty in keeping the lambs on feed the levels were dropped to 1 and 1.75 mg. on the 28th day of the trial. The latter group was raised to 2.5 mg. on the 49th day of the trial. Feed consumption improved with the initial reduction in level of stilbestrol fed, and did not seem to be affected by the increase made on the 49th day.

Discussion

The primary interest of the animal nutritionist in the use of synthetic sex hormones or hormone-like compounds is in their effects in producing more rapid gains and more economical use of feed in the production of safe and wholesome meat. That these effects are not easily obtained in lambs without decreasing carcass quality or altering the reproductive organs, mammary glands or other tissues or organs of the body has been well summarized by Andrews *et al.* (1956).

Thus far, the beef steer is in a class by itself in giving a large and consistent response in rate of gain and in feed efficiency at levels of stilbestrol feeding that produce relatively unimportant side effects of an undesirable nature. This effect, for the steer, was first shown by Burroughs *et al.* (1955).

Stilbestrol stimulated weight gains (P=.05) in ewe lambs in only one of four trials and at a level of 3.5 mg. per lamb per day. This level had proved somewhat toxic to similar ewe lambs fed during the same winter period (Feb.-May) a year earlier. A level of 2 mcg. of stilbestrol per pound of feed failed to stimulate gains as it had for Hale *et al.* (1954) in two out of three trials with wether lambs. A level of 2 mg. per ewe lamb daily was without effect in Experiment 3, caused a significant depression in gains in Experiment 4, and increased gains slightly, though not significantly, in Experiment 5. Acker *et al.* (1955) fed .5 mg. of stilbestrol per pound of feed (approximately 2 mg. per head) and increased gains in lambs of both sexes. Light *et al.* (1956) report that levels of .5, 1.0 or 1.5 mg. of stilbestrol per lamb daily produced a significant improvement in rate of gain independent of the sex of the lamb. Jordan (1956) found that 2 mg. per lamb daily had no effect on rate of gain. These results seem less inconsistent if one considers the gains made by the control lambs in the various experiments in which stilbestrol was used orally. Stilbestrol-fed lambs, produced significantly greater gains than did controls that gained in the range of .23 to .35 lb. per head per day (Acker *et al.*, 1955; Light *et al.*, 1956; Smith *et al.*, 1956). Slightly lower levels of stilbestrol were reported to have less than a significant effect by Jordan, (1955) in a trial in which the control lambs gained .40 lb. per day. In the trials being reported, stilbestrol fed at levels from 2 to 3.5 mg. per head daily failed to stimulate gains significantly over gains by control lambs of either .44 or .48 lb. per head daily. On the other hand, in the present experiments, the 3.5 mg. level produced significantly greater gains than control lambs that gained at the rate of only .36 lb. per head per day. Oddly enough, ewe lambs, in Experiment 4, that gained significantly less than the control lambs, gained at the same rate (.42 lb. per day) as did those lambs, in Experiment 5, that gained significantly more than their controls. In experiment 5, the 2 mg. level of stilbestrol stimulated exactly one-half the increase in daily rate of gain over the control lambs as did the 3.5 mg. level; however, Hale *et al.* (1955) found that the 2 mg. level was superior in all respects to the 3.5 mg. level for wether lambs. This comparison suggests that maximum stimulation in rate of gain in ewe lambs is dependent upon a greater daily intake of stilbestrol than is true for the wether lamb. Other effects which were graded by level of stilbestrol fed were an increase in the percentage of yearling carcasses, an increase in uterine size, a decrease in weight of the ovaries, and a retardation of follicle development. The lowering of carcass grade was independent of size of dose if only the 2 and 3.5 mg. levels of stilbestrol are considered.

The work with the androgenic compounds, methyl testosterone and testosterone propionate, is difficult to explain in that a statistically significant stimulation in rate of gain was produced in only one of four trials. The injection of an oil suspension of testosterone propionate produced growth, but lack of finish and hypertrophy of the thyroid; whereas the

feeding of 42.9 mg. of methyl testosterone per lamb daily produced more finish than the basal ration, no evidence of growth stimulation and had no effect on the weight of the thyroid (Experiment 1). Burris *et al.* (1953) reported a significant increase in thyroid weight and in weight gains for both steers and heifers from the injection of testosterone during the fattening period. The feeding of methyl testosterone and stilbestrol, or the injecting of testosterone propionate into lambs being fed stilbestrol prevented stilbestrol from exerting its usual effect in making the pelts difficult to remove. The same observation has been reported by O'Mary *et al.* (1952) for implanted testosterone propionate.

The progesterone-estradiol implant, with modifications, may have possibilities for the future since the ewe lambs so treated in Experiment 4 gained 15% more than the control lambs and were 16% more efficient in the use of feed, but the ratio of 250 mg. of progesterone to 10 mg. of estradiol was unsatisfactory. This particular ratio and dose apparently produced both growth and aging simultaneously. The growth was apparent in greater gains, but less finish and the aging was apparent in that 50% of the lambs yielded carcasses which were classed as yearlings because of whiteness of the bones and complete ossification of the break joint. The same observation has been made by Acker *et al.* (1955). Then too, the treatment stimulated sexual excitement and the mating reaction, which Turner (1949) suggests depends upon an optimal progesterone-estradiol ratio, as demonstrated in the guinea pig. It should be realized that the lambs used in this experiment were old-crop lambs fed during February to May and were approaching the age at which one would expect a percentage of yearling carcasses to be produced on any good fattening ration; however, no such carcasses were present in the untreated control lambs. Perhaps this hastening of maturity prompted by progesterone and/or estradiol is more fully expressed in ewe lambs than it is in wethers.

Summary

Results from three individual and two group-feeding experiments involving 324 Western ewe lambs are reported.

An injection of 3.3 mg. of testosterone propionate per 100 lb. live weight per day increased rate of gain in ewe lambs by 38% (significant at P=.05) and increased feed intake over control lambs in one experiment, but neither this injected dose nor a 30 mg. pellet implant had any effect on gains in three subsequent trials. Methyl testosterone, fed at levels of either 8.4 or 42.9 mg. per lamb daily, produced a significant increase in rate of gain in Experiment 1, but the higher level was without effect on gains in subsequent trials. Carcass grades were highest in the group fed the higher level of methyl testosterone and lowest in the group injected with testosterone propionate in the trial in which these androgens significantly increased rates of gain. When effective in stimulating gains, testosterone propionate increased the size of the thyroids. Shorn lambs implanted with 30 mg. of

testosterone propionate gained 27% faster than their wooled pen mates, but failed to gain significantly faster than either shorn or wooled control lambs.

An implant of 250 mg. of progesterone plus 10 mg. of estradiol increased rate of gain significantly, improved feed efficiency, and increased the daily consumption of corn, but not of total feed. This treatment lowered carcass grades significantly and 50% of the carcasses were classed as yearlings. Excessive riding was evident by the fourth day after implantation and 20% of the lambs accepted a vasectomized ram at least once during the first half of a 71-day trial.

The feeding of 3.5 mg. of stilbestrol per ewe lamb daily produced significantly greater gains (Experiment 5), but this level could not be tolerated by similar ewe lambs in Experiment 4. Feeding 2 mg. of stilbestrol per lamb daily had no effect (Experiment 3), reduced gains significantly (Experiment 4) and increased gains by an amount just short of significance in Experiment 5. Actual carcass grades (lamb basis) were lowered, but not significantly, by either 2 or 3.5 mg. of stilbestrol per lamb per day and the percentage of yearling carcasses increased with the level of stilbestrol fed.

The progesterone-estradiol implant increased uterine size significantly and reduced the size and number of follicles, but had no effect on the weight of the ovaries. Stilbestrol increased uterine size, decreased ovary size and retarded follicle development; whereas testosterone propionate had no significant effect on any of these measurements, but tended to increase ovary weights and number of developing follicles.

Literature Cited

Acker, Duane, J. V. Whiteman, W. D. Gallup and A. D. Tillman. 1955. Effect of certain hormones on feed digestibility feedlot performance, and carcass quality of lambs. Okla. Agr. Exp. Sta. 29th Annual Livestock Feeders' Day. Misc. Pub. No. MP-43.

Andrews, F. N., W. M. Beeson and C. Harper. 1949. The effect of stilbestrol and testosterone on the growth and fattening of lambs. J. Animal Sci. 8:518.

Andrews, F. N., Martin Stob, T. W. Perry and W. M. Beeson. 1956. The effect of oral and subcutaneous estrogen and androgen administration on growth and carcass quality of lambs. J. Animal Sci. 15:575.

Bell, T. Donald, Walter H. Smith and A. B. Erhart. 1954. The effect of stilbestrol upon lamb performance in the feedlot. J. Animal Sci. 13:425.

Bell, T. Donald and A. B. Erhart. 1956. Feedlot and pasture fattening tests with feeder lambs. Livestock Feeders' Day Report. Kansas Agr. Exp. Sta.

Burris, Martin J., Ralph Bogart and Hugo Krueger. 1953. Alteration of activity of the thyroid gland of beef cattle with testosterone. Proc. Soc. Exp. Biol. Med. 84:181.

Burris, Martin J., Ralph Bogart, A. W. Oliver, Andrea Overman Mackey and G. E. Oldfield. 1954. Rate and efficiency of gains in beef cattle. Ore. Agr. Exp. Sta., Tech. Bul. 31.

Burroughs, Wise, C. C. Culbertson, Edmund Cheng, W. H. Hale and Paul Homeyer. 1955. The influence of oral administration of diethylstilbestrol to beef cattle. J. Animal Sci. 14:1015.

Clegg, M. T. and H. H. Cole. 1954. The action of stilbestrol on growth response in ruminants. J. Animal Sci. 13:108.

Clegg, M. T., Reuben Albaugh, Joseph Lucas and W. C. Weir. 1955. A comparison of effects of stilbestrol on the growth response of lambs of different age and sex. J. Animal Sci. 14:178.

Galloway, J. H., L. J. Bratzler, J. H. Blakesler and J. Meiter. 1952. Quarterly Bul. Mich. Agr. Exp. Sta. 35:68.

Hale, W H., C. D. Story, C. C. Culbertson and Wise Burroughs. 1953. The value of low levels of stilbestrol in the rations of fattening lambs. J. Animal Sci. 12:918.

Hale, W. H., P. G. Homeyer, C. C. Culbertson and Wise Burroughs. 1955. Response of lambs fed various levels of diethylstilbestrol. J. Animal Sci. 14:909.

Johnston, E. F., Frank Hudson, Ralph Bogart, A. W. Oliver and Fred. R. McKenzie. 1956. The effect of injected testosterone on adult ewes. J. Animal Sci. 15:177.

Jordan, R. M. 1950. The effect of stilbestrol on fattening lambs. J. Animal Sci. 9:383.

Jordan, R. M. 1953. Effect of level of stilbestrol on growth and fattening of lambs. J. Animal Sci. 12:680.

Jordan, P. S., R. M. Jordan and H. G. Groom. 1955. Effect of stilbestrol, progesterone-estradiol implants and oral administration of stilbestrol on fattening lambs. J. Animal Sci. 14:936.

Jordan, P. S., R. M. Jordan and H. G. Groom. 1956. Effect of chlortetracycline, stilbestrol and chlortetracycline-stilbestrol supplements on fattening lambs. J. Animal Sci. 15:188.

Klosterman, E. W., W. E. Dinusson, Merle R. Light and M. L. Buchanan. 1951. The value of A. P. F. and stilbestrol for growing and fattening lambs. North Dakota Bi Monthly Bulletin. 14:43.

Light, Merle R., W. E. Dinusson, R. M. Richard and D. W. Bolin. 1956. Urea and stilbestrol for fattening lambs. J. Animal Sci. 15:570.

Means, T. M., F. N. Andrews and W. M. Beeson. 1953. The effects of hormones on the growth and fattening of lambs. J. Animal Sci. 12:176.

Pope, A. L., C. C. O'Mary, W. E. Batherman, R. W. Bray and L. E. Casida. 1950. The effect of certain steriod hormones on the growth, fattening and carcass quality of heavy lambs. J. Animal Sci. 9:680.

O'Mary, C. C., A. L. Pope, G. D. Wilson, R. W. Bray and L. E. Casida. 1952. The effects of diethylstilbestrol, testosterone, and progesterone on growth and fattening and certain carcass characteristics of western lambs. J. Animal Sci. 11:656.

Richard, R. M. and W. E. Dinusson. 1954. Effect of stilbestrol and bacitracin on growing and fattening lambs. North Dakota Bi Monthly Bulletin. 16:86.

Smith, Walter H., T. Donald Bell, A. B. Erhart and D. L. Mackintosh. 1955. The effect of hormone administration on feed lot performance, carcass qualities and reproductive organs of feeder lambs. J. Animal Sci. 14:1256. (Abst.).

Turner, C. Donnell. 1949. General Endocrinology. Philadelphia. W. B. Saunders Co.

ESTROGENIC ACTIVITY OF FECES AND URINE FOLLOWING ORAL ADMINISTRATION OF DIETHYLSTILBESTROL TO LAMBS

C. D. Story, E. W. Cheng, John Pauls and W. H. Hale [1]
Iowa Agricultural Experiment Station

THE value of diethylstilbestrol (stilbestrol) in the rations of fattening steers for increased gain and improved feed efficiency has been demonstrated by Burroughs *et al.* (1954) (1955) and Perry *et al.* (1955). Similar responses with lambs have also been noted by Hale *et al.* (1955), Bell and Erhart (1956) and Light *et al.* (1956). Although stilbestrol has received wide acceptance in supplements for fattening cattle very little is known about its mode of action or metabolism in ruminants.

Nearly all of the work on excretion pathways of synthetic estrogens has been conducted with laboratory animals given single doses of the compounds. Wilder and Williams (1948) recovered 35% of the stilbestrol in the urine of rabbits given large single oral doses. Jellinck (1954) using the rabbit recovered from 8 to 20% of a single injected dose of stilbestrol in the urine and up to 50% in the feces. Hanahan *et al.* (1951) found from 77 to 98% of the radioactivity of a single injected dose of labeled stilbestrol in the intestinal tract of rats 6 hours after injection. Similar findings were reported for the mouse and dog given radioactive stilbestrol (Twombly and Schoenewaldt, 1951). Recently Turner (1956) has shown that feces from cows receiving 10 mg. of stilbestrol per day contained as much estrogenic activity as did the feed fed. These data suggest that a large proportion of the orally administered stilbestrol appears in the feces.

The purpose of the study reported in this paper was to determine the amount of stilbestrol in the feces and urine of lambs being fed different levels of stilbestrol.

Experimental

Four wether lambs each weighing about 80 lb. were used in this study and fed in the experiment as follows: Period 1, a basal ration containing no stilbestrol; period 2, the basal ration plus 1 mg. of stilbestrol per lamb per day; period 3, the basal ration plus 2 mg. of stilbestrol per lamb per day; period 4, the basal ration without stilbestrol. Within each period the lambs were on the experimental ration for 2 weeks prior to a one-week, pre-collection and the one-week collection periods. Collections were made by usual techniques using a bag for fecal collections while the lambs were in metabolism crates equipped with urine collection pans. All lambs were handled individually during all periods. During the pre-collection and col-

[1] Journal paper No. J-3049 of the Iowa Agricultural Experiment Station, Ames. Project No. 869.

lection periods the lambs were fed at a 1¼ maintenance level in order to secure complete and constant food intake. The basal ration was formulated as follows: ground alfalfa hay 50, cracked corn 33, cane molasses 15 and soybean oil meal 2. The stilbestrol was mixed with the ration in amounts to supply the 1 and 2 mg. levels.

Total fecal collections were made for each lamb. The 7-day dried collection for each lamb was mixed and a 10% aliquot taken. The aliquots from each of the 4 lambs were put together and thoroughly mixed. This mixed sample of feces was ground and several sample taken for a composite sample to be assayed. Total urine collections were made for each lamb. Of the daily excretion from each lamb 10% was retained. The daily aliquots for the four lambs were mixed together for the 7-day collection period and dried on a steam plate. The composite dried sample was used for assay.

Assays for estrogenic activity of the feces and urine were conducted in a manner similar to that described by Preston *et al.* (1956). Five mice were used per replicate. Preliminary assays were made on the feces and urine of lambs that had received different levels of stilbestrol in order to estimate estrogenic activity of the material to be assayed. The material to be assayed was then added to the mouse basal ration at such a level that the expected uterine response would fall within the range of the standard assay curve. The feces and urine from the first three periods were assayed at one time using the same standard mouse assay curve, whereas the feces and urine from the fourth period were assayed using a different standard mouse assay curve. Consideration was also given to the possibility of conjugated stilbestrol in the urine and feces of the treated lambs that might not be detected by the mouse assay technique. In the preliminary assays, acid hydrolyzed urine and feces were checked along with nontreated samples. The assays failed to reveal an increase in estrogenic activity due to acid hydrolysis. Consequently, the assays were carried out on untreated samples.

Results and Discussion

The results of the mouse assays of the urine and feces from the four experimental periods are shown in table 1. It can readily be seen that no estrogenic activity was present in the urine or feces during the period the lambs were on the basal ration alone. During the two test periods considerable estrogenic activity was observed in both urine and feces as noted by the uterine response of mice. Material collected during period 4 contained little if any estrogenic activity.

Table 2 presents the excretion of the fed stilbestrol in the feces and urine of the lambs during periods 2 and 3. Assays of the rations showed approximately 0.9 mg. of stilbestrol fed per lamb per day during period 2 rather than the intended level of 1 mg. and slightly more than 2 mg. during period 3. If both levels of stilbestrol feeding are taken into consideration

approximately 48% of the stilbestrol was recovered in the feces and 32% in the urine, making a total recovery of 80%. It is believed that the techniques used in this study are sufficiently sensitive so that the unrecovered stilbestrol represents a true loss of the compound, provided the active material excreted has the same rate of activity as the compound fed. The

TABLE 1. ESTROGENIC ASSAY OF URINE AND FECES FROM LAMBS FED STILBESTROL

Period	Addition to 1 gm. mouse basal ration	Uterine wt. (mg.)			Estimated stilbestrol potency
		Rep. 1	Rep. 2	Mean	
Assay curve	none	4.7	7.3	6.0	
for periods 1, 2 and 3	.005[a]	10.3	7.5	8.9	
	.010	21.9	23.9	22.9	
	.020	41.8	32.7	37.7	
	.040	53.9	62.6	58.2	
Period 1. No stilbestrol					
Feces	40 mg.	4.7	5.8	5.3	
Urine	40 mg.	5.1	4.6	4.9	
Period 2. 1 mg. stilbestrol per lamb per day					
Sheep ration	4 mg.	10.8	9.7	10.3	.432 mg. DES per lb.
Sheep ration	8 mg.	19.6	19.5	19.6	ration fed
Feces	2 mg.	8.3	11.9	10.1	1.036 mg. DES per lb.
Feces	4 mg.	18.7	18.1	18.4	dry feces
Urine	2 mg.	15.6	16.5	16.0	4.0γ DES per gm. dry
Urine	4 mg.	42.8	34.2	38.5	urine
Period 3. 2 mg. stilbestrol per lamb per day					
Sheep ration	2 mg.	10.2	10.6	10.4	1.026 mg. DES per lb.
Sheep ration	4 mg.	27.8	24.9	26.4	ration fed
Feces	1 mg.	10.3	11.6	11.0	2.518 mg. DES per lb.
Feces	2 mg.	23.3	29.3	26.3	dry feces
Urine	1 mg.	20.4	25.1	22.8	9.3γ DES per gm.
Urine	2 mg.	34.5	40.5	37.5	dry urine
Assay curve	none	7.8	6.7	7.3	
for period 4	.005[a]	13.2	14.5	13.8	
	.010	20.8	16.9	18.9	
	.020	28.7	25.9	27.3	
	.040	48.6	47.0	47.8	
Period 4. No stilbestrol					
Feces	20 mg.	7.2	10.4	8.8	
Feces	40 mg.	9.5	8.5	9.0	
Urine	15 mg.	7.8	8.8	8.3	
Urine	30 mg.	10.7	8.3	9.5	

[a] Mcg. of diethylstilbestrol.

fate of the remaining 20% is unknown. Apparently it is not stored in the tissues of animals fed stilbestrol (Preston *et al.* 1956; Turner, 1956). The most likely explanation would be degradation by rumen microorganisms or metabolic degradation of absorbed stilbestrol. The data from period 4 suggest that the compound is not stored in the tissue and released after feeding has ceased. One of the main pathways of excretion of injected stilbestrol is by way of the bile. Whether this pathway is of importance for orally administered stilbestrol is not known, but seems likely. These

TABLE 2. EXCRETION OF STILBESTROL BY EXPERIMENTAL LAMBS

Items	Dry matter	Stilbestrol	Percent of stilbestrol intake
	gm.	mg.	
	1 mg. stilbestrol per lamb per day		
Total intake per lamb	5,090	5.98	
Total fecal output per lamb	1,346	3.07	51.3
Total urine excretion per lamb	368	1.50	25.1
	2 mg. stilbestrol per lamb per day		
Total intake per lamb	5,171	14.18	
Total fecal output per lamb	1,153	6.40	45.1
Total urine excretion per lamb	596	5.53	39.0

data also suggest that the larger the amount of stilbestrol fed to lambs, the greater the excretion in the urine.

Summary

Four lambs were placed on metabolism studies and fed two different levels of stilbestrol. At the 1 mg. level per lamb per day, 51% of the stilbestrol appeared in the feces and 25% in the urine. At the 2 mg. level, 45% appeared in the feces and 39% in the urine. Combining both levels of feeding, 80% of the fed stilbestrol was recovered in the urine and feces. The possible fate of the remaining 20% is briefly discussed.

Literature Cited

Bell, T. Donald and A. B. Erhart. 1956. Feedlot and pasture fattening tests with feeder lambs. Livestock Feeders Day Report. Kansas Agr. Exp. Sta.

Burroughs, Wise, C. C. Culbertson, Joseph Kastelic, Edmund Cheng and W. H. Hale. 1954. The effect of trace amounts of diethylstilbestrol in rations of fattening steers. Science 20:66.

Burroughs, Wise, C. C. Culbertson, Edmund Cheng, W. H. Hale and Paul Homeyer. 1955. The influence of oral administration of diethylstilbestrol to beef cattle. J. Animal Sci. 14:1015.

Hale, W. H., P. G. Homeyer, C. C. Culbertson and Wise Burroughs. 1955. Response of lambs fed varied levels of diethylstilbestrol. J. Animal Sci. 14:909.

Hanahan, Donald J., E. G. Daskalakis, T. Edwards, Hyp. J. Dauben, Jr. and Richard W. Meikle. 1951. Observations on metabolism of C^{14}-diethylstilbestrol. Arch. Biochem. Biophy. 33:342.

Jellinck, P. H. 1954. The excretion of synthetic oestrogens. Biochem. J. 58:262.

Light, Merle R., W. E. Denussion, R. M. Richard and D. W. Bolin. 1956. Urea and stilbestrol for fattening lambs. J. Animal Sci. 15:570.

Perry, T. W., W. M. Beeson, F. N. Andrews and Martin Stob. 1955. The effect of oral administration of hormones on growth rate and deposition in the carcass of fattening steers. J. Animal Sci. 14:329.

Preston, Rodney, Edmund Cheng, C. D. Story, Paul Homeyer, John Pauls and Wise Burroughs. 1956. The influence of oral administration of diethylstilbestrol upon estrogenic residues in the tissues of beef cattle. J. Animal Sci. 15:3.

Turner, C. W. 1956. Biological assay of beef steer carcass for estrogenic activity following the feeding of diethylstilbestrol at a level of 10 mg. per day in the ration. J. Animal Sci. 15:13.

Twombly, Gray H. and Erwin F. Schoenewaldt. 1951. Tissue localization and excretion routes of radioactive diethylstilbestrol. Cancer 4:296.

Wilder, Smith, A. E. and P. C. Williams. 1948. Urinary elimination of synthetic oestrogens and stilbestrol glucuronide in animals. Biochem. J. 42:253.

OBSERVATIONS ON PARATHYROIDECTOMIZED GOATS [1, 2]

VEARL R. SMITH, G. H. STOTT AND C. W. WALKER [3]

University of Wisconsin

MOST textbooks state that herbivores are more tolerant of parathyroidectomy than carnivores. Few studies have been made on ruminants from which the parathyroids have been removed. MacCallum and associates (1907) thyroparathyroidectomized eight goats (two mature, six kids) and five mature sheep. Both mature goats showed some stiffness and muscular twitchings but no acute symptoms of tetany. The results on the kids were variable; some showed symptoms of parathyroid deficiency, but others did not. Only slight twitching was observed in two of the five sheep.

Simpson (1913) thyroparathyroidectomized four adult ewes and four lambs 5 to 7 weeks of age. No symptoms of parathyroid deficiency were observed during the 3 to 4 months the ewes were under observation. All four lambs exhibited symptoms of tetany and died within a short time after the operation. In a later study Simpson (1921) thyroparathyroidectomized four ewes, thyroidectomized one ewe, and used one ewe as control. All the ewes were pregnant. There were no acute symptoms of tetany. One of the thyroparathyroidectomized ewes died; it was carrying two dead fetuses. Another one aborted a dead fetus and showed tetany after the abortion. The other two ewes lambed normally. At autopsy a small nodule resembling parathyroid tissue was found in one of the normal-lambing ewes. In these early studies on parathyroidectomy, no observations were made on chemical constituents of the blood. Campbell and Turner (1942) parathyroidectomized two goats and noted a marked decrease in the blood serum calcium; however, tetany was not observed until the animals were subjected to a low calcium ration.

This study was undertaken to obtain more complete information on the reaction of ruminants to parathyroidectomy.

Experimental Procedure

Six kid goats, 8 to 9 months of age, were thyroparathyroidectomized. Two other kid goats, from which only the thyroids were removed, served as control. The thyroids and external parathyroids were removed from three mature goats that were four months pregnant and the thyroids were removed from two other mature goats in the same stage of pregnancy.

Because of the possible variation in position of the internal parathyroids within the thyroids, the thyroids were completely removed. The external parathyroids, located near the bifurcation of the carotid arteries, could

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.
[2] This study was supported by funds from Babson Bros., Co., Chicago, Illinois.
[3] Department of Dairy Husbandry, Madison.

usually be distinguished from lymph nodes by their red color. In young animals they were within the anterior lobes of the thymus, adjacent to the carotid artery. In some kids the anterior portion of the thymus was removed and the parathyroids were located only after the thymus had been carefully dissected. Because of the lack of the thymus in the mature goats, the parathyroids were more easily located. All tissue removed was identified histologically.

Preoperative blood samples were drawn from the jugular of all animals to establish serum calcium and plasma inorganic phosphorus levels. Blood samples were drawn daily for the first few days after the operation and at less frequent intervals thereafter. The methods of analyses were the same as those used in previous studies (Smith and Merrill). The animals were maintained on a ration of corn silage, alfalfa hay, and a concentrate made up of 500 lb. rolled oats; 250, cracked corn; 150, wheat bran; 100, soybean meal; 10, bone meal and 10, trace mineral salt.

Seventy-four days after the operation, the goats still alive (one died before this time) were randomly divided into two groups. Four goats were placed on a low calcium ration and three remained on the original ration.

For 3 weeks the low calcium group was fed ad libitum wheat straw and a concentrate mixture consisting of 60 lb. corn; 25, corn gluten meal; and 15, wheat bran. This mixture provided a calcium-phosphorus ratio of 1:6.9.

Results

Table 1 shows the effect of thyroparathyroidectomy and thyroidectomy on blood serum calcium and plasma inorganic phosphorus of kid goats. The blood serum calcium declined considerably below the preoperative level by the second or third day after the operation in the thyroparathyroidectomized goats. There was considerable variation among individuals. Serum calcium declined in the thyroidectomized goat, but the decline was not of the same magnitude as in the others. The serum calcium leveled off much below normal after the initial decline. An exception to this was goat P84. On the 52nd day after the operation, her blood calcium had dropped from 8.5 mg. % of the previous week to 6.2 mg. Five days later the animal was found dead. At no time during this phase of the study did the kid goats exhibit acute symptoms of tetany. Occasionally some of them showed stiffness of the legs and had difficulties in getting up and down.

Four days after the goats were subjected to the low calcium ration, P83 showed symptoms of tetany. The neck and legs were stiff and the goat moved with great difficulty. Also, the animal was having difficulty in defecating and urinating. Two days later P74 developed the same symptoms, but with less severity. There was a further decline in serum calcium concomitant with the symptoms of tetany. Nineteen days after being subjected to the low calcium, P83 was observed in severe tetany. The

TABLE 1. CHANGES IN THE SERUM CALCIUM AND PLASMA INORGANIC PHOSPHORUS AS A RESULT OF THYROIDECTOMY AND THYRO-PARATHYROIDECTOMY OF KID GOATS

(Values in mg. %)

Goat No.	P74		P77 [a]		P80		P83 [c]		P73		P76 [a]		P84 [b]		P85	
Days	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
Pre-operative																
28	10.8				11.4		12.0				12.2		10.6		10.9	
20	12.2	6.0			11.7	6.5	12.2	4.3	11.6	4.9	11.7	6.7	11.8	5.3	11.9	4.9
5	11.7	4.0			11.5	5.2	11.5	3.5	12.2	5.9	12.5	4.3	11.6	5.3	12.3	5.3
Post-operative																
1	9.9	4.2			8.4	5.0	9.1	6.2	9.3	2.6	10.2	6.3	8.1	6.1	9.7	5.6
2	8.1	6.1			8.8	6.7	8.6	6.8	8.0	7.0	10.1	7.0	6.9	7.7	7.4	7.2
3	6.4	6.3			7.5	5.2	6.8	5.8	7.2	4.2	10.6	5.1	6.9	7.2	7.0	6.6
4	6.3	4.7			8.2	4.8	8.1	3.7	7.7	4.9	10.3	5.9	5.7	6.2	6.3	6.7
7	6.4	5.3			9.5	4.1	8.6	3.9	8.4	4.8	10.9	6.5	6.0	6.3	6.8	5.1
10	6.7	3.4			7.8	3.7	7.3	3.0	7.5	4.3	10.3	5.1	6.8	3.6	7.5	4.7
17	8.2	2.6			9.1	3.7	7.4	3.0	8.7	4.8	10.8	3.5	6.4	2.2	10.4	3.8
24	9.2	2.7			9.2	5.7	7.4	5.9	9.3	3.2	10.4	4.8	7.4	2.2	8.7	3.8
31	7.8	3.4			8.4	5.1	7.6	5.2	8.6	4.2	10.8	7.8	7.2	4.0	8.9	3.5
38	7.4	3.0			8.1	4.9	8.6	4.6	9.1	5.0	10.4	6.3	7.6	3.2	9.0	4.2
45	8.0	4.7			8.4	4.7	8.2	4.1	8.9	3.9	10.8	5.5	8.9	3.9	8.8	5.0
52	8.1	2.8			7.6	3.0	8.3	2.9	8.4	2.9	10.4	3.0	8.5	6.3	8.1	3.7
59	6.6	5.4			8.3	5.1	9.6	3.0	10.5	5.0	11.2	5.7	6.2	...	8.4	5.5
66	7.0	4.5			9.4	...	6.7	...	7.4	5.3	10.0	7.2			8.5	...
73	7.1	3.0			8.0	2.1	7.8	3.1	7.7	3.4	11.2	5.1			8.6	6.4
	Subjected to low Ca ration								Continued on original ration							
74	5.7	3.8	10.7	7.0	7.6	4.2	5.6	7.5	8.6	2.8	11.0	3.8			7.3	7.5
81	5.3	3.6	10.3	8.4	6.4	4.8	4.9	6.8	7.9	4.6	10.7	5.6			6.8	8.2
88	5.7	5.6	10.8	7.2	7.1	6.0	5.2	11.8	8.6	6.5	10.4	5.2			6.1	13.3
	Returned to original ration															
95	5.7	4.9	11.0	5.4	9.4	4.4			8.2	5.7	11.1	5.2			5.5	12.1
102	6.8	5.8	11.2	5.7	9.4	5.6			9.2	3.5	11.5	3.0			7.0	8.5
109	6.4	5.4	11.3	6.9	9.5	4.5			9.3	4.1	11.1	5.3			6.6	12.0
116	7.0	4.2	10.9	6.2	9.1	3.0			9.4	3.1	11.1	4.2			5.8	10.8

[a] Thyroidectomized control.
[b] Died on day 57. Blood sample taken before death.
[c] Died on day 86. Blood sample taken before death.

goat was lying on her side with all four legs stiff and extended. She was frothing at the mouth, breathing was labored and with a respiratory crow. A blood sample was taken and an i.v. infusion of calcium gluconate attempted, but the goat died during the infusion. There was some increase in the serum calcium level when the goats were switched back to the original ration.

There were no consistent changes of the plasma inorganic phosphorus. Many species of animals show an elevated phosphorus simultaneous with

TABLE 2. CHANGES IN THE SERUM CALCIUM AND PLASMA INORGANIC PHOSPHORUS AS A RESULT OF THYROIDECTOMY, THYRO-PARATHYROIDECTOMY AND PARTURITION
(Values in mg. %)

Goat No.	33[a]		151[a]		24		152		143	
Days	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
Preoperative										
1	10.8	6.6	9.8	4.5	9.6	5.8	10.8	6.5	10.0	6.3
Postoperative										
1	8.6		8.9	4.5	5.4	4.8	6.4	4.6	5.6	2.4
2	8.7	5.7	8.4	1.6	4.8	1.8	5.9	3.3	4.7	1.9
3	9.2	7.1	8.1	3.7	4.6	4.7	6.3	4.8	5.1	2.0
5	9.7	6.5	9.0	6.4	4.9	3.4	5.1	4.5	4.9	6.5
7	9.0	5.0	10.0	4.3	5.8		5.0	1.6	4.4	4.1
8					6.5[b]					
9					4.9	3.2	3.4	1.6	4.9	5.1
11	10.5	4.7			5.3	2.5			5.4	3.3
14			8.9	3.4						
16							4.3	2.6[b]		
18	8.9	3.1								
21	9.3	7.1[b]		5.0[b]	5.3	2.0	5.1	2.9	4.8	2.4
22	9.8		8.7	6.2						
24	9.9	5.0								
28	9.8	9.3	9.8	6.2	5.1	3.7	6.0	4.0	4.8	1.8
35	10.2		10.1	4.2			7.8	2.1	4.5	2.3
38									5.5	2.6[b]
40									5.0	4.2
42	9.0	7.9					8.3	2.2		

[a] Thyroidectomized control.
[b] Day of parturition.

the decline of calcium after parathyroidectomy. P83 had an increase in P from 3 mg. % the day before being subjected to the low calcium ration, to 11.8 mg. % the day of death. Its phosphorus increased markedly the 86th day after the operation without any marked change in serum Ca. Goats P74 and P85 died 145 and 217 days, respectively, after having the parathyroids excised. The other kid goats were alive 8 months after the operation.

Results on pregnant goats are presented in table 2. Both the concentration of calcium and phosphorus in the blood of the completely thyroparathyroidectomized goats, 24, 143, 152, decreased precipitously after the operation. By the second day calcium levels were approximately one-half the normal level and continued low until after parturition, when they

[a] Died on day 86. Blood sample taken before death.

began to rise slowly. The low concentration of phosphorus is contrary to that reported for parathyroidectomized animals of other species.

Serum calcium declined 1–2 mg. % the day after the operation in the two thyroidectomized goats, 33 and 151. This level persisted for 3 days, then gradually increased, reaching pre-operative levels by the end of the first week. Plasma phosphorus declined on the second day after the glands were removed, then increased and varied over a wide but normal range.

The thyroparathyroidectomized goats did not show symptoms of parathyroid deficiency until the second day after the operation when all three became stiff and had difficulty in getting up and lying down. This condition disappeared 24 hours later and the three animals behaved normally until the end of the first week. At that time, goat 24 had aborted a dead fetus and the other two were in acute tetany. Calcium gluconate was administered i.v. to the two goats in tetany, and within the hour the tetanic symptoms disappeared. It was necessary to continue the calcium gluconate treatment daily until a short time before parturition, when symptoms of parathyroid deficiency disappeared. During parturition and afterwards there were no outward symptoms of parathyroid deficiency nor were there any symptoms of milk fever. The kids of 152 and 143 were born dead but apparently fully developed. Of interest was the lack of mammary development prior to and following parturition in the three thyroparathyroidectomized goats.

The two thyroidectomized goats were normal as far as outward appearance was concerned following the operation. There was the usual udder development prior to parturition and each gave birth to twins without complications. Milk production was below that of the previous year for both animals.

Discussion

The classical reaction to parathyroidectomy is a decline in serum calcium and an elevation of the plasma inorganic phosphorus. In contrast to some other species, excision of the parathyroid brought no consistently marked changes in the plasma inorganic phosphorus of the kid goats. In fact, with pregnant goats a decline of the plasma inorganic phosphorus generally occurred concomitant with the decline in serum calcium. These results are in accord with Lotz, Talmage and Comar (1954) who found that injections of parathyroid hormone into sheep caused an elevation of the plasma inorganic phosphorus. In rats, dogs and man a depression of the phosphorus normally occurs after injections of parathyroid extract. However, Robinson, Huffman, and Burt (1927) reported a decrease in plasma inorganic phosphorus of another ruminant, the calf, to which parathyroid extracts had been administered.

There was no udder development preparatory for lactation in the three thyroparathyroidectomized goats. Although attempts were made to initiate lactation by twice daily milking after parturition only a scanty flow was

established. Folley and associates (1942) and Cowie (1945) demonstrated that parathyroidectomy greatly restricted milk flow in rats. Munson *et al.* (1954) also showed that lactation after parathyroidectomy was decreased but the percent of calcium in the milk was unaffected; however, intact rats subjected to a low calcium ration produced the expected quantity of milk but the calcium content of the milk was 30% below normal. Manunta and Mureddu (1951) report an increase in the calcium content of ewes' milk as the result of injections of parathyroid extract.

Summary

Serum calcium and plasma inorganic phosphorus determinations were made on kids and on pregnant goats that were thyroidectomized or thyroparathyroidectomized. In both the kid and mature goats, thyroparathyroidectomy resulted in a marked drop in the serum calcium but no marked change of the plasma inorganic phosphorus. Subjecting the kid goats to a low calcium ration resulted in a further decrease of the serum calcium. Four of the six thyroparathyroidectomized kids died from parathyroid deficiency.

The pregnant goats showed tetany within a week after removal of the parathyroids. After parturition no further symptoms of parathyroid deficiency were observed. Lactation in the thyroparathyroidectomized goats was nil.

Literature Cited

Campbell, I. L. and C. W. Turner. 1942. The relation of the endocrine system to the regulation of calcium metabolism. Mo. Agr. Exp. Sta., Res. Bul. 352.

Cowie, A. T. and S. J. Folley. 1945. Parathyroidectomy and lactation in the rat. Nature. 156:719.

Folley, S. J., H. M. Scott-Watson, and E. C. Amoroso. 1942. Further experiments on lactation in thyroidectomized rats: The role of the parathyroids. J. Endocr. 3:178.

Lotz, W. E., R. V. Talmadge, and C. L. Comar. 1954. Effect of parathyroid extract administration in sheep. Proc. Soc. Exp. Biol. Med. 85:292.

MacCallum, W. G., H. S. Thomson, and J. B. Murphy. 1907. Tetany after parathyroidectomy in herbivora. Bul. Johns Hopkins Hosp. 18:333.

Manunta, G., and F. Mureddu. 1951. Rapport fra calcio ematico e calcio nel latte nella pecoro. Ach. fisiol. 51:1.

Munson, P. L., S. U. Toverud, and A. D. Kenny. 1954. Restriction of dietary calcium intake during the period of molar calcification in the suckling rat. J. Dental Res. 33:676.

Robinson, C. S., C. F. Huffman, and K. L. Burt. 1927. The effect of the administration of parathyroid extract on normal calves. J. Biol. Chem. 73:477.

Simpson, S. 1913. Age as a factor in thyroidectomy in sheep. Quart. J. Exp. Physiol. 6:119.

Simpson, S. 1921. Thyroparathyroidectomy in the sheep. Am. J. Physiol. 55:281.

Smith, V. R., and W. G. Merrill. 1954. Parturient paresis VII. A study of the leucocytes of cows with parturient paresis. J. Dairy Sci. 37:967.

FACTORS AFFECTING THE THYROID UPTAKE OF I^{131} IN DAIRY COWS [1, 2, 3]

E. W. SWANSON, F. W. LENGEMANN [4] AND R. A. MONROE [5]
University of Tennessee [6]

RADIOACTIVE iodine, I^{131}, is now being used widely in medical clinics to assist in the evaluation of thyroid function. The rate of uptake and the percentage of I^{131} accumulated by the thyroid gland in 24 hours have been accepted as good indications of thyroid activity. The application of this test to dairy cows has been reported by Blincoe and Brody (1955) and Swanson *et al.* (1954). Blincoe and Brody (1955) found that the rate of thyroid uptake of I^{131}, maximum thyroid I^{131} content, and the I^{131} release rate indicated changes in thyroid activity due to environmental temperature consistent with the expected effects and with changes in energy metabolism. Since thyroid function is an important factor in growth and milk production, a simple method for its determination would be valuable in dairy production investigations. The tests reported here were undertaken to determine the effect of lactation and season of the year upon the thyroid uptake of I^{131}. Other complicating factors also have been investigated.

Experimental Methods

After some preliminary trials the following methods were adopted for these studies. The cow to be tested was dosed by intravenous catheter with 100 microcuries of carrier-free NaI^{131}. A quantity of the dosing solution equal to 20% of the dose was diluted in a 50 ml. flask for a counting standard. Counts of radioactivity were made with a specially constructed sodium iodide crystal scintillation counter (Nuclear) connected to a standard count rate meter (Nuclear). The crystal was held a constant 8 in. from the neck by means of a brass shield. The extension of lead shielding around the crystal was reduced to decrease the variation in counting due to slight changes in movement of the cow or the crystal. Thyroid counts were made by holding the shield against the neck of the cow in the thyroid region in such a manner that a maximum count rate was registered. This count was corrected for background by taking a count

[1] This manuscript is published with the approval of the Director of the University of Tennessee Agricultural Experiment Station, Knoxville.

[2] The radioactive materials used in this work were obtained from the Oak Ridge National Laboratory on allocation from the United States Atomic Energy Commission.

[3] This work was completed under Contract No. AT-40-1-GEN-242 between the University of Tennessee, College of Agriculture, and the Atomic Energy Commission.

[4] Present address: University of Tennessee, Chemistry Department, Memphis.

[5] Present address: Poultry Department, Cornell University, Ithaca, N. Y.

[6] Department of Dairying, Knoxville, Tennessee, and Atomic Energy Commission Agricultural Research Program, Oakridge, Tenn.

with the shield held against the shoulder of the cow. The standard solution was counted and corrected for normal background each time thyroids were checked. All thyroid counts were expressed as (thyroid/standard) times 0.2, which has been called "percentage of the I^{131} dose in the thyroid." The repeatability of the method was satisfactory since it produced constant results at any one period in the hands of several different technicians.

The cows used in this study were one pair of grade Jersey identical twins 3 years old, two registered Jersey cows 4 and 6 years old, two grade Jersey cows about 5 years old, and a pair of grade Guernsey identical twins 4 years old. One of the cows was dry and not pregnant during the study. Another cow was kept non-pregnant and lactating throughout the study. In the first year one set of identical twins consisted of a dry and a lactating member, but the next year they were both lactating. The second pair of identical twins consisted of one dry and one lactating. Tests were started with one pair of twins in November, 1953; two other cows were included in March, 1954; two others were added in April, 1954; and the second pair of twins was first dosed in January, 1955. Counts were made at about six week intervals, from March, 1953, to June, 1955. This time interval allowed the previous tracer dose to have decayed or have been metabolized so that no thyroid count was detectable just prior to the next dose.

The hay fed was mostly mixed hay grown at Oak Ridge, but some purchased hay reported to have come from Wisconsin was also fed. No grazing was allowed. After March, 1954, all grain fed to these cows was a 16% protein mixture composed of grain and grain by-products fortified with one percent common salt and one percent dicalcium phosphate as the only minerals. An iodine analysis of this grain mix showed that it contained 0.36 p.p.m. It has been called a low iodine ration in contrast to a commercial dairy feed used previously and for other cows which contained 2.6 p.p.m. of iodine. Grain was fed to lactating cows according to milk production and to the dry twin of the second pair at the same rate as her mate. The cows were stabled in box stalls with access to a small outside pen at all times.

Results

In a previous experiment with identical twins (Swanson *et al.*, 1954) it was noted that maximum accumulation of I^{131} by the thyroid was about twice as great in November as in May, about the same in September as in May, and lowest of all in July. This suggested that season and especially prolonged high temperatures as encountered in June and July, 1953, markedly affected thyroid activity. Since the heifers were dry in November and fed very little or no grain, but lactating and fed grain in the other periods, a lactation or feed effect may have been added to the seasonal effect. The data from the present experiments have been arranged to determine the importance of these factors affecting I^{131} uptake by the thyroid.

The average activity of the thyroid with time after dosing as observed in these studies is shown in figure 1. This is an average of 69 observations taken from 8 different cows in 12 periods in which the low-iodine grain mix was used. There was a rapid concentration of I^{131} in the thyroids during the first two days with a further increase to the peak uptake on the third day. For the fourth, fifth, and sixth days there was little change in radioactivity of the thyroid. The rate of decrease from the sixth to the 14th day was roughly linear on both arithmetic and logarithmic scales. The average daily loss of I^{131} from the thyroid was 0.5% of the dose, and

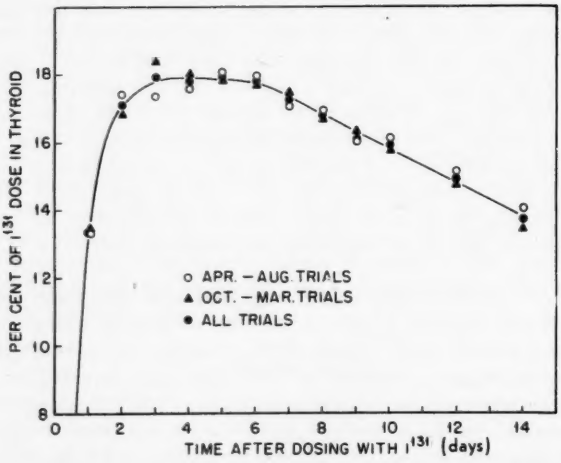

Figure 1. The average uptake and release by the thyroids of dairy cows of radioactive iodine tracer doses, representing 8 cows, 12 different tests, and 69 observations made at all seasons of the year.

the average daily rate of I^{131} release was 3.05% of the thyroid content. The peak uptake was 18.0% of the dose. Differences between the average of the 31 April to August (summer) values and the 38 October to March (winter) values are also shown in figure 1. The winter peak of 18.4% uptake was reached on the third day, but the summer peak of 18.0% was not reached until the fifth day. The average daily loss from the sixth to the 14th day was 0.54% of the dose in winter and 0.48% in summer, and the rates of thyroid I^{131} release were 3.38% in winter and 2.76% in summer. The differences observed were in the direction expected; i.e., slower uptake and release rate in summer than in winter, but they were so small that their practical significance is very doubtful. The difference between the summer and winter curves is not statistically significant.

The possibility of differences in thyroid uptake of I^{131} due to lactation

was first studied with the Jersey identical twins which had previously shown nearly identical thyroid activity when managed alike. The curves of iodine uptake and release for these cows at different periods are shown in figure 2. While No. 18 was dry her thyroid uptake of I^{131} was distinctly higher than that of No. 19 which was milking. When both were dry, their uptake and release curves were again very similar. The next year while both were lactating, their thyroid I^{131} curves remained nearly identical (as shown by the two bottom pairs of curves in figure 2). While twins 18

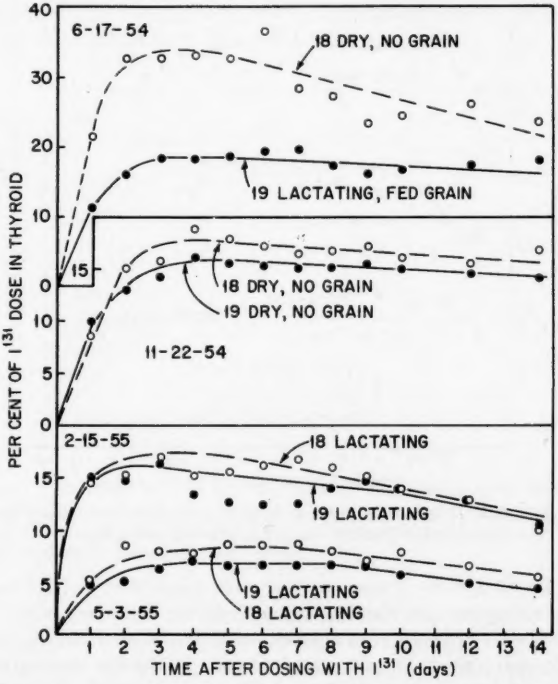

Figure 2. The influence of lactation and/or feed upon the thyroid concentration of a tracer dose of radioactive iodine by identical twin dairy cows.

and 19 were dry they were not fed grain. In spite of the fact that a low-iodine mixture had been fed 19, there was suspicion that it was affecting the thyroid uptake of I^{131}.

Identical twins 20 and 21 were then tested with No. 20 lactating and No. 21 dry and when both were fed the same ration, including grain in equal amounts. The results of three tests with these twins are shown in figure 3. In each period the thyroid I^{131} uptake and release curves for the

two cows were very similar. The I^{131} concentration in the thyroid of 21 was consistently higher than 20 only in the first test at which time 20 had been fed grain for one week longer than 21 prior to the test. Again the main differences, which might indicate a higher thyroid activity in a dry than a lactating cow, may be explained by grain feeding.

The effect of a very small amount of iodine in the feed of the cow upon I^{131} accumulation in the thyroid is shown in figure 4, which depicts the

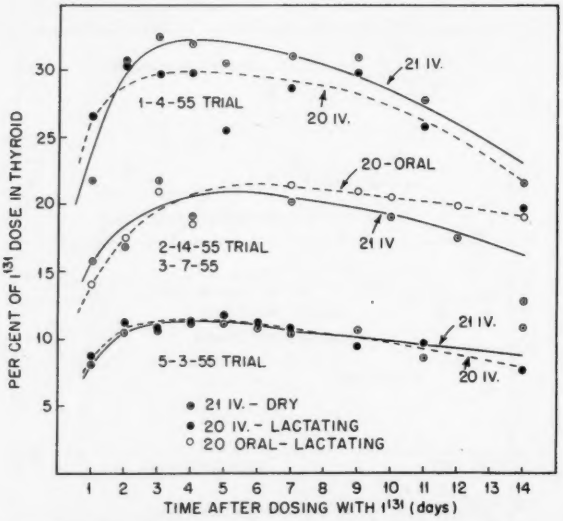

Figure 3. A comparison of the thyroid uptake of radioactive iodine by dry vs. lactating identical twin dairy cows fed alike.

average thyroid activity values for two cows which were fed a commercial grain feed one year and the low-iodine feed the next year. On the low-iodine feed their thyroid accumulation of I^{131} followed nearly the same pattern as that shown for all cows in figure 1. On the commercial feed the maximum thyroid concentration of I^{131} was only 7.5% of the dose and the disappearance rate was very low, amounting to only 0.5% in 10 days. The cows were getting 10 to 12 mg. iodine daily from the commercial feed compared to about 1.5 mg. from the low-iodine grain mixture. These results show the need for very low and carefully controlled iodine intakes if comparable thyroid activity of cows is to be measured by I^{131} uptake.

The effect of stage of lactation upon thyroid activity is shown in figure 5. The average thyroid counts for three, four and five days after dosing each of the lactating cows at the various periods are plotted beginning at the first period before parturition. The data shown in figure 5 indicate a

tendency for an increase in thyroid activity just after freshening. During the next three to four months a continuous downward trend in thyroid I^{131} uptake was noted, with the lowest point in the lactation reached at 4 to 5 months. After this point there was wide variation in the changes observed, with an average trend indicating a plateau of thyroid activity slightly below the prepartum level and slightly above the low mid-lactation level. Four of these cows calved in December and January, and the others in May and August. A seasonal effect may have complicated these stages of lactation data, but it is not to be expected that the seasonal trend from May to August would be toward increased thyroid I^{131} uptake as the data from the fifth to the eighth month indicate (figure 5).

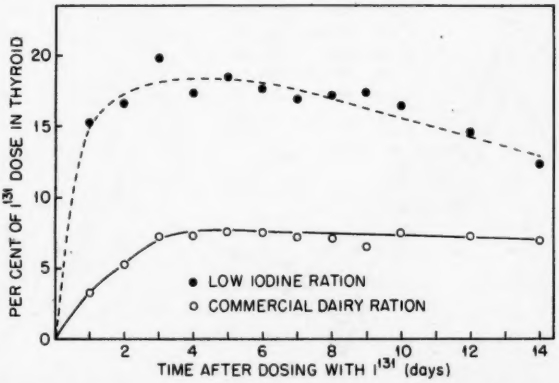

Figure 4. The effect of an iodine-supplemented commercial dairy ration furnishing 10–12 mg. I per day compared to a non-supplemented grain ration furnishing 1.5 mg. I per day upon thyroid concentration of tracer doses of radioactive iodine in lactating dairy cows.

The average peak I^{131} uptake by the thyroids (3, 4 and 5-day values averaged) of all cows is presented in figure 6 according to the time, in successive months (1954–5), that the tests were made. To depict possible seasonal effects uncomplicated by lactation or grain feeding, the data for two dry cows during the first year are shown in a separate graph. The trends of values of all cows and the two dry cows were very similar; so the differences observed were probably not affected by lactation, but were due to other factors affecting the tests at each of the respective periods. The peak values in June, 1954, the low values in May and June, 1955, and rather constant values from July to November, 1954, do not fit the hypothesis that season has a major effect upon the I^{131} binding power of the thyroid.

Three cows were used in a study of the effect of thyroxine administration

upon thyroid I^{131}. Cow 19 was dosed for a routine test June 16, 1954, and beginning June 20 was fed 100 mg.l-thyroxine along with her grain feed each day. Thyroid changes of this cow were nearly the same as those exhibited at the same time by 5 cows not fed thyroxine. Thyroxine feeding at this level did not prevent release of thyroid I^{131} during the first 10 days. After feeding cow 19 l-thyroxine for 113 days, she was dosed for another test on October 11, and thyroxine feeding stopped. No I^{131} appeared in her thyroid for the next 7 days; so she was given the same dose as the original on October 18. After this dose a slow uptake was noted which

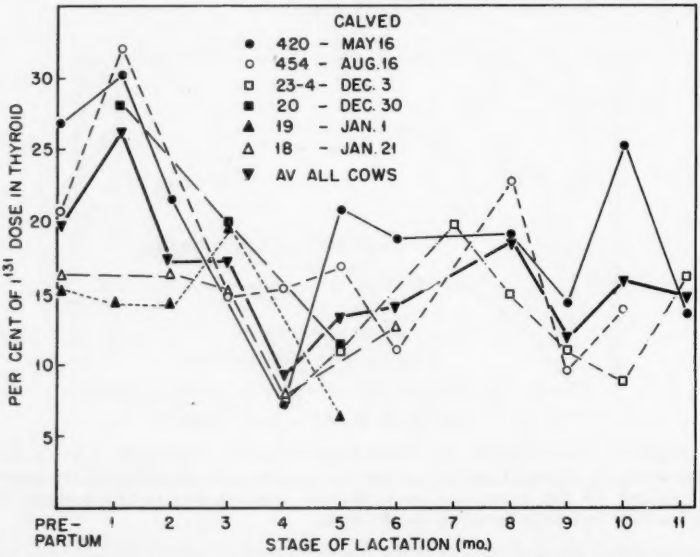

Figure 5. The peak uptake of radioactive iodine by thyroids of dairy cows according to the stage of lactation at which tests were made.

reached a peak at about 7 days. This indicated that the thyroid had not returned to normal function within 2 weeks after stopping thyroxine feeding.

Five days before the June, 1955 test lactating cow 19 and dry cow 420 were each given daily subcutaneous injections of 5 mg. l-thyroxine. These were continued until 3 days after dosing when the amount of thyroxine injected daily was reduced to 3 mg. and continued for 12 more days. The peak thyroid uptake of I^{131} by the lactating cow was 6.0% and it occurred on the third day after dosing. The peak uptake by the dry cow was 4.6% on the second day. The average peak uptake of 3 non-treated cows dosed at that time was 14.3%. The thyroxine injections had decreased expected

peak uptakes more than 50%. The thyroid I^{131} contents on the fourteenth day were 5.3% and 2.6% of the dose for the lactating and dry cows, respectively, showing that some net release of I^{131} had occurred even with the l-thyroxine injections.

Discussion

This series of thyroid I^{131} uptake tests with dry and lactating cows over a period of nearly 1.5 years has revealed that several factors including

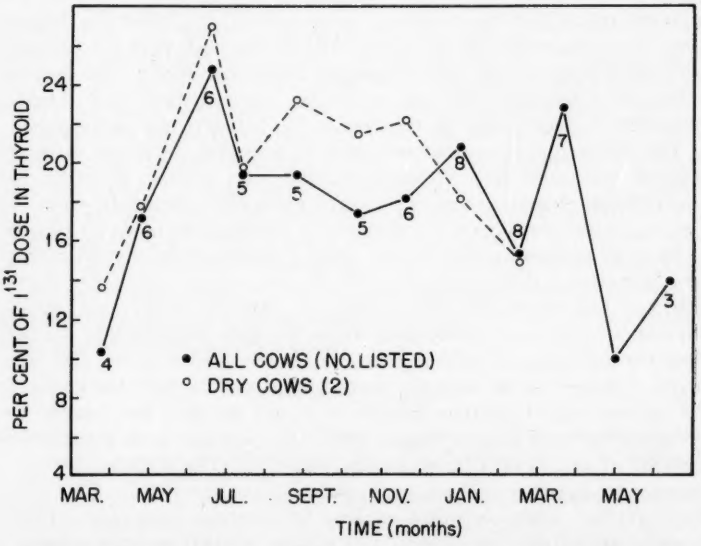

Figure 6. The average peak uptakes of radioactive iodine by thyroids of dry and lactating dairy cows according to the times and seasons that the tests were made.

iodine intake, stage of lactation, and season affect the results obtained. The greatest effect was due to iodine in the feed, which prevented normal I^{131} uptake and release by the thyroid. This effect was quite pronounced with a commercial dairy ration which contained added iodine and other trace minerals. It was also suggested from the difference in uptake between grain-fed and non-grain-fed identical twins that a special low iodine feed contributed enough iodine to depress the I^{131} uptake of the thyroid (figure 2). It was also noted that cows which were tested just after moving to Oak Ridge invariably had higher thyroid I^{131} uptakes than in later trials whether fed grain or not. The progressive decline in thyroid I^{131} uptake of

cows 20 and 21 (figure 3) may have been due to such a change in feeding (both were fed grain) as well as to the changes in season.

The changes due to lactation were relatively minor. With most of the cows tested there was evidence of peak thyroid activity at the peak of lactation which was followed by a decline to the lowest thyroid activity at mid-lactation. These observations do not follow the known effect of lactation on metabolism. They do agree with the usual change at about mid-lactation in which the cow stops losing weight and begins to gain steadily (Turner, 1924). The results with dry and lactating identical twins as well as the comparisons between dry cows and all cows at all tests indicate that lactation per se does not produce a measurable effect. Differences between cows were quite marked in most tests. These differences were not associated with differences in milk yield or average lactational ability. The fact that values for identical twins were so similar indicates that some inherited physiological factor may be responsible for between cow differences.

The effects due to season were hard to interpret. Selection of certain pairs of tests could show a marked seasonal effect in either direction. It is believed that seasonal changes in iodine metabolism definitely occur, but that occasional measurement of thyroid I^{131} uptake alone is not an adequate index of all of the changes. The I^{131} binding power of the thyroid may not be a sensitive indicator of the need of the cow for iodine for thyroid activity. The observation of Seiden and Fenger (1913) that summer thyroids contain more iodine than winter thyroids suggests that the body does not maintain a steady equilibrium between iodine stores and iodine needs. Changes due to seasonal temperatures may be reflected in thyroid I^{131} uptake only at the time changes occur and not after the changes have persisted for more than a month. Such an effect has been suggested by Leblond *et al.*, (1944), from results with rats. The definite correlation between temperature changes and thyroid uptake reported by Blincoe and Brody (1955) when controlled ambient temperatures were changed every 4 weeks shows that the thyroid I^{131} uptake of the cow is responsive to temperature changes.

The observation that the thyroid of a cow which had been fed thyroxine 113 days was not back to normal I^{131} uptake two weeks after stopping thyroxine was interesting in comparison with a similar test previously reported (Swanson *et al.*, 1954) which showed normal thyroid activity 30 days after stopping *Protamone* feeding. These results and the minor changes in thyroid uptake up to 10 days after thyroxine feeding started show that thyroid uptake responses to thyroxine feeding are relatively slow.

The average peak thyroid uptake of I^{131} of about 18% of the dose observed in these studies is lower than normal values of 25 to 50% reported for humans (Haigh *et al.*, 1954) and the values of 62% reported by Blincoe and Brody (1955) for Jersey cows, and 45.6% reported by Lewis *et al.*, (1955) for Jersey calves. The actual content of I^{131} in the thyroid of one cow sacrificed three days after dosing was found by Monroe *et al.*,

(1954) to be only 11.7%, with this cow having excreted in urine, feces and milk 43.7, 17.6 and 7.2% of the dose, respectively. The rate of release of I^{131} from the thyroid found in this study agrees with that reported by Blincoe and Brody (1955) in spite of wide differences in maximum uptakes. Differences in counting technique will affect release rate values less than peak uptake values.

Summary

Studies of I^{131} uptake by the thyroid gland of 8 Jersey and Guernsey cows during a period of nearly 18 months have been reported. I^{131} in the thyroid of the cow reached a maximum of about 18% of the dose at 3 to 5 days after dosing, and after 6 days it was released at a constant rate of about 3% per day. By suitable comparisons it has been shown that thyroid activity reaches a maximum in the first few weeks after parturition but that in later lactation thyroids of dry cows are just as active as those of lactating cows fed the same feed. Iodine in amounts usually included in dairy rations was found to markedly inhibit uptake of I^{131} by the thyroid, and some evidence that a low-iodine grain ration also inhibited thyroid I^{131} uptake was observed. Effects which could be attributed to seasonal temperatures were noted, but the average differences found between seasonal measurements were relatively unimportant. Measuring thyroid activity of cows by this method on an uncontrolled survey basis could not be expected to provide reliable comparable data.

Literature Cited

Blincoe, C. and S. Brody. 1955. The influence of ambient temperature, air velocity, radiation intensity, and starvation on thyroid activity and iodine metabolism in cattle. Mo. Agr. Exp. Sta. Res. Bul. 576.

Blincoe, C. and S. Brody. 1955. The influence of diurnally variable temperatures on the thyroid activity and iodide metabolism of Jersey and Holstein cows. Mo. Agr. Exp. Sta. Res. Bul. 579.

Haigh, C. P., M. Reiss and J. M. Reiss. 1954. Measurements of absolute radio-iodine uptake in the assessment of human thyroid activity. J. Endocrinology 10:273.

Leblond, C. P., J. Gross, W. Peacock and R. D. Evans. 1944. Metabolsm of radio-iodine in the thyroids of rats exposed to high or low temperatures. Am. J. Physiol. 140:671.

Lewis, R. C., E. P. Reineke and J. R. Lodge. A technique for estimating the thyroxine secretion rate of dairy cattle. J. Animal Sci. 14:1250. 1955.

Monroe, R. A., C. L. Comar and E. W. Swanson. 1954. Unpublished data partially summarized in UT-AEC Program Progress Report ORO–133.

Seiden, A. and F. Fenger. 1913. Seasonal variation in the iodine content of the thyroid gland. J. Biol. Chem. 13:517.

Swanson, E. W., R. A. Monroe and C. L. Comar. 1954. Using identical twin dairy cows to determine the effect of iodinated casein (Protamone) on milk production, thyroid activity, and body weight changes. J. Dairy Sci. 37:659.

Turner, C. W. 1924. A study of the relation between feed consumption and milk secretion. J. Dairy Sci. 7:535.

EFFECT OF EXPOSURE TO HIGH ENVIRONMENTAL TEMPERATURE AND SHEARING ON SEMEN PRODUCTION OF RAMS IN WINTER [1]

R. H. Dutt and Paul T. Hamm
University of Kentucky, Lexington

THE studies of McKenzie and Berliner (1937) and Comstock *et al.* (1943) suggest that high environmental temperatures are responsible for the lowered quality of ram semen during summer months. Phillips and McKenzie (1934) have shown that heat applied locally to the scrotum caused testicular derangement. McKenzie and Berliner (1937) also demonstrated a deleterious effect on semen quality after the scrotum of the ram had been insulated, or after the entire body had been exposed to elevated temperatures. Dutt (1954) found that low conception rate in ewes bred early in the breeding season (August and September) was due principally to failure of fertilization. Later Dutt and Bush (1955) and Dutt and Simpson (1954) found that fertility rate in ewes was greatly improved when the rams were kept in an air-conditioned room at 45–48° F. during the summer months. Hamm (1954) found that a period of six weeks elapsed before semen of rams placed in an air-conditioned room at 65° F. in mid-summer was comparable in quality to that from rams which had been kept in an air-conditioned room all summer. It is of interest to determine the period of time necessary for recovery of normal semen production in rams following spermatogenic impairment as a result of whole body exposure to elevated air temperature.

In the present study sheared and unsheared rams were exposed to elevated air temperature during the season of the year when spermatogenesis is normally at a high level. Certain physiological reactions of the rams were studied in addition to semen production.

Experimental Procedure

Six purebred mature Southdown rams, all of proven breeding capacity, were randomly assigned to three groups during the first week of January, 1955. Group 1, unsheared and maintained conventionally, served as the control. Group 2 sheared and Group 3 unsheared were placed in a heated room on January 12 and kept for a week at 90° F. and relative humidity 60–65%. The experimental temperature was selected on the basis of results reported by McKenzie and Berliner (1937). Air velocity in the room one foot above the floor varied from 20 to 80 cu. ft. per minute in different parts of the room. Treatment completed, the experimental groups were

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

placed with the control group in an unheated open room free from drafts.

Rectal temperature, pulse rate and respiration rate were obtained on all rams as follows: (a) On three alternate days during the control period. (b) On fifth, sixth and seventh days of the experimental period. Rectal temperature was taken with a 4-inch clinical thermometer fully inserted; a stethoscope was used for pulse rate; and respiration rate was based on flank movements. All measurements were made between 2 and 3 p.m. on the respective days.

Semen was collected from all rams by means of an artificial vagina on three occasions at two-day intervals during the pretreatment period. Volume of semen, percent motile cells, percent morphologically abnormal cells and sperm cell concentration were determined for each ejaculate. Semen volume was measured to the nearest 0.1 ml. Initial motility was estimated by examining a drop of semen diluted with Krebs solution under a high power microscope. Percent motile cells was estimated at 10-percent class intervals; in all cases the estimates were made within 10 minutes of collection. Concentration of sperm cells was determined by counting on a hemacytometer chamber. Percentage of morphologically abnormal cells was calculated by counting the abnormally-shaped cells in the same fields on the hemacytometer from which cell concentration was computed.

Subsequent to the week of treatment, semen was collected semi-weekly from all rams for a period of nine weeks. Laboratory studies were conducted on only one of the two collections per ram per week.

Results

Treated rams did not appear to suffer any gross ill effects as a result of elevated environmental temperature. None went off feed during the heat treatment. Increased respiration rate revealed considerable heat stress but it was not severe enough to cause panting or gasping of breath.

Physiological Responses. Table 1 presents changes in rectal temperature, pulse rate and respiration rate for the control and two experimental groups in the preliminary period and week of treatment. It may be observed that rectal temperature increased in both experimental groups. The increases were statistically significant. Rectal temperature in the unsheared rams (105° F). was considerably above normal as reported by Clawson (1928) and by Ritzman and Benedict (1931). Outside air temperature during the preliminary period ranged from 37° to 55° F. and during the experimental week from 41° to 55° F.

Pulse rate in the sheared group increased seven beats per minute whereas it decreased by one in the unsheared group. These changes are consistent with the observations of Regan and Richardson (1938) who reported a decrease in pulse rate in cows as environmental temperature increased.

Changes in respiration rate were quite pronounced in both groups, especially so in the unsheared group. These increases reflect a physiological attempt to maintain normal body temperature through heat loss in expelled

TABLE 1. AVERAGE RECTAL TEMPERATURE, PULSE RATE AND RESPIRATION RATE OF CONTROL AND EXPERIMENTAL RAMS [a]

Group	Treatment	Rectal temp. F.	Pulse rate per min.	Resp. rate per min.
1. Control-unsheared	Prelim. week	102.4	71	35
	Exper. week [b]	102.2	73	34
2. Experimental-sheared	Prelim. week	101.8	69	25
	Exper. week	102.4[c]	76[c]	134[c]
3. Experimental-unsheared	Prelim. week	102.0	69	23
	Exper. week	105.0[c]	68	178[c]

[a] Average for 2 rams at 2 p.m. on three successive days. Experimental room maintained at 90° F. and relative humidity 60–65%.
[b] Continued as in preliminary week without treatment.
[c] Differences between periods within ram groups tested significant at 1% level.

air. Increased rectal temperature in the unsheared rams indicates that they had reached the upper limits of their ability to maintain normal body temperature through increased respiration rate.

Semen Characters. Volume of semen in the experimental groups revealed little or no change. Treatment also had no observable effect on libido or sexual drive.

Average percentage of motile sperm cells for the three groups during the control period and weekly averages for nine weeks after the treatment are shown in figure 1. Throughout the study the average initial motility for the control group ranged between 75 and 85% motile cells. During the control period the average percentage of motile cells ranged between 80

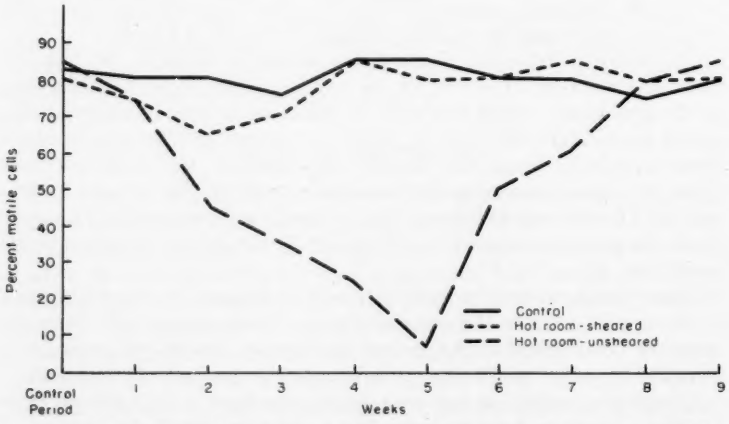

Figure 1. Weekly averages of percent motile cells after exposure of rams for one week at 90° F

and 85 for the three groups. Motility declined only slightly after the treated groups had been in the heated room for about a week. Thereafter, sperm cell motility for the unsheared group declined rapidly until five weeks after the elevated-temperature treatment, at which time motility had dropped to less than 10%. At the sixth week the percentage of motile cells had increased and eight weeks after the treatment there was no difference in percentage of motile cells between the control and the unsheared groups.

During the fourth, fifth and sixth weeks after the treatment the percentage of motile cells for the unsheared group was significantly lower than that of the control group. At no time was there any significant difference in percentage of motile cells between the control and the sheared

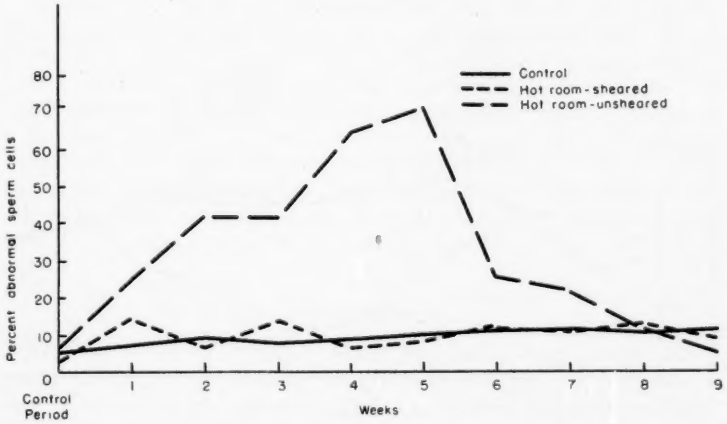

Figure 2 Weekly average of percent abnormal sperm cells after exposure of rams for one week at 90°F

groups. Motility of sperm cells from the sheared rams was only slightly lowered by the elevated temperature. The lowest average for this group was recorded on the second week after the treatment, when 65% of the cells were estimated to be motile.

One measure of semen quality is percentage of morphologically abnormal cells. Throughout this study abnormal cells in the semen of the untreated rams ranged from 5 to 12%. During the control period the range in abnormal cells was from 3.0 to 7.5% for all three groups (figure 2). At the end of the week in the heated room, semen from the unsheared rams averaged 25% abnormal cells, and five weeks after the treatment 70% of the cells in this group were classified as morphologically abnormal. Percent abnormal cells in this group then decreased, and by the eighth week had dropped to 10%, which was approximately the same as the control group at that time.

For the third, fourth, fifth and sixth weeks, percent abnormal cells in the semen from the unsheared group was either significantly or highly significantly greater than that of the control group. Semen from the sheared rams did not exhibit any great increase in abnormal sperm cells. At the end of the week in the heated room, this group averaged 14.5% abnormal cells, which was the highest throughout the study. At no time was there any great difference in percentage of abnormal cells between the sheared rams and the control rams (figure 2). Most of the abnormals consisted of tailless cells and cells with crooked or coiled tails.

Sperm cell concentration for the period studied is shown in figure 3. Concentration for the control group throughout the entire study varied from

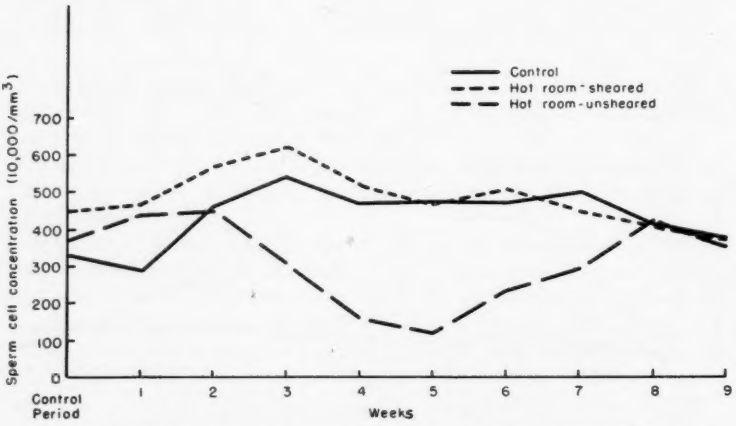

Figure 3. Weekly averages of sperm cell concentration after exposure of rams for one week at 90°F.

3 to 5.4 billion cells per ml. For the three groups during the control period the range was from 3.2 to 4.5 billion cells per ml. Five weeks after treatment concentration in the unsheared rams had dropped to 1.2 billion cells per ml. Eight weeks after treatment concentration for this group had increased to 4.2 billion cells per ml. The treatment did not cause any drop in sperm cell concentration for the sheared group during any part of the study.

Discussion

Shearing of rams previous to exposure in a heat chamber reduced heat stress as measured by changes in body temperature, pulse rate and respiration rate in comparison with the responses in unsheared rams under similar treatment. Data presented in table 1 demonstrate the effectiveness of shear-

ing in the loss of metabolic heat in rams subjected to an ambient temperature of 90° F. and relative humidity ranging from 60 to 65%.

Semen studies revealed that elevated body temperature impaired spermatogenic activities. A decline in semen quality was evident in the second week after treatment in the form of sperm cell degeneration which became most severe in unsheared rams five weeks after treatment. Young (1927) demonstrated that spermatocytes and spermatids were the first cells to be affected by the application of temperature to the scrotum of guinea pigs. That the earlier stages in spermatogenesis were most severely damaged in treated rams is indicated by lowest motility and highest percentage of abnormal cells five weeks after treatment because the supply of older stages was reduced by repeated ejaculations subsequent to treatment.

Semen quality of unsheared experimental rams did not return to the level of untreated rams until an interval of eight weeks had elapsed. Hamm (1954) had previously shown that quality semen was not produced in rams transferred in mid-summer to the experimental room maintained at 65° F. and relative humidity 70 to 80% until after a lapse of six weeks.

No tests of fertilizing capacity of sperm were conducted in the present study. But, since spermatogenic activity is intimately related to body temperature it is desirable to maintain rams under conditions whereby the deleterious effects of high summer temperatures may be minimized. The current study confirms the importance of shearing rams in the summer months.

Summary

Six rams of proven breeding ability were randomized equally into a control group and two experimental groups. One of the latter was sheared and the other was left unsheared. Both experimental groups were placed in a heated room at 90° F. and relative humidity 60 to 65%, for one week in the month of January. Physiological responses during the experimental period and subsequent changes in semen characters may be summarized as follows:

1. Body temperature showed a more pronounced increase in unsheared rams than in sheared.
2. Pulse rate increased in the sheared rams but was not affected appreciably in the unsheared.
3. Respiration rate showed a marked increase in both experimental groups, being higher in the unsheared.
4. Volume of semen changed very little as a result of treatment.
5. Motility of sperm was affected adversely, especially in the unsheared group which showed the lowest percentage of motile cells during the fifth week after treatment.
6. Abnormal cells increased in both experimental groups, especially in the unsheared which attained their maximum in the fifth week after treatment.

7. Sperm cell concentration decreased more appreciably in the unsheared than in the sheared rams.
8. Semen quality of all treated rams was normal after eight weeks.

Literature Cited

Clawson, A. B. 1928. Normal rectal temperatures of sheep. Am. J. Physiol. 85:251.

Comstock, R. E., W. W. Green, L. M. Winters and H. V. Nordskog. 1943. Studies of semen and semen production. Minn. Tech. Bul. 162.

Dutt, R. H. 1954. Fertility rate and embryonic death loss in ewes early in the breeding season. J. Animal Sci. 13:464.

Dutt, R. H. and Leon F. Bush. 1955. The effect of low environmental temperature on initiation of the breeding season and fertility in sheep. J. Animal Sci. 14:885.

Dutt, R. H. and E. C. Simpson. 1954. Environmental temperature and fertility of Southdown rams early in the breeding season. J. Animal Sci. 13:1019.

Hamm, Paul T. 1954. Effect of environmental temperature on semen characteristics and physiological reactions of Southdown rams during summer months. Master's Thesis, University of Kentucky.

McKenzie, F. F. and Victor Berliner. 1937. The reproductive capacity of rams. Mo. Agr. Exp. Sta. Res. Bul. 265.

Phillips, R. W. and F. F. McKenzie. 1934. The thermo-regulatory function and mechanism of the scrotum. Mo. Agr. Exp. Sta. Res. Bul. 217.

Regan, W. M. and G. A. Richardson. 1938. Reactions of the dairy cow to changes in environmental temperature. J. Dairy Sci. 21:73.

Ritzman, E. G. and F. G. Benedict. 1931. The heat production of sheep under varying conditions. New Hampshire Agr. Exp. Sta. Tech. Bul. 45.

Young, W. C. 1927. The influence of high temperature on the Guinea Pig testes. J. Exp. Zool. 49:459.

OVULATION, PRENATAL MORTALITY AND LITTER SIZE IN SWINE [1]

EARL L. LASLEY
Iowa State College

THE number of ova released in a single estrus greatly exceeds the number of pigs born (Hammond, 1914; 1921; Corner, 1923; Robertson *et al.*, 1951; and Squiers *et al.*, 1952). Casida (1952) re-evaluated previous estimates and concluded that the number of young born (including stillbirths) was approximately 56% of the number of corpora lutea. Since litter size is the number of ova released minus the number of eggs not fertilized and the number of zygotes dying before birth, it seemed desirable to inquire into the relative importance of genetic and nongenetic factors affecting these traits.

Materials and Methods

Observations were made on 87 sows born in August or September of 1952. Each sow had farrowed two litters and was again pregnant when slaughtered during the summer of 1954. Five different kinds of breeding, as listed in table 1, were included. The Poland Chinas were pure-breds. In the other groups the breed of sire is listed first. If not a first cross, the breed of maternal grandsire is listed second, etc.

Immediately after weaning their second litter, the sows were penned with four boars in a single lot. This was done to provide maximum fertilization while randomizing the effects of sires. The data obtained at slaughter included corpora lutea count, number of dead fetuses and number of live fetuses. These data were considered to characterize the sows, rather than the fetuses.

Slaughter was intended to be at about the 80-day stage of gestation but, as individual breeding dates were not obtained, the day of slaughter had to be determined by considering the individual sow's condition and the date when she had been penned with the boars. After slaughter, the actual stage of gestation was estimated by comparing the crown-rump length of fetuses with the prenatal growth curve of Warwick (1928). The number of observations summarized by stage of gestation are: under 50 days, 16; 51–70 days, 12; 71–90 days, 40; and over 90 days, 19. Although variation in stage of gestation was large it is not believed to bias seriously the estimates of genetic parameters because most mortality occurs during fertilization and implantation (Squiers, *et al.*, 1952) and because there is little reason to

[1] Journal paper No. J-2874 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 1127. The project is cooperative with the U.S.D.A., A.R.S., Animal & Poultry Husbandry Research Branch, Regional Swine Breeding Laboratory, Ames.

TABLE 1. MEANS BY BREEDING GROUPS [a]

Breeding group	No. of sows	N_1	N_2	N_O	N_A	N_D	Percent mortality
Poland China	19	6.6	7.8	15.2	9.9	5.3	35
Landrace-Poland	8	10.9	12.1	16.5	9.0	7.5	45
Poland-Landrace	13	10.2	12.0	18.2	10.6	7.6	42
Duroc-Poland-Landrace	39	10.0	12.3	16.3	9.6	6.7	41
Montana No. 1-Duroc-Poland-Landrace	8	10.6	11.2	17.1	9.4	7.7	45
All sows	87	9.4	11.2	16.4	9.7	6.7	41

[a] N_1 is the number of pigs born in first litter including stillbirths.
N_2 is the number of pigs born in second litter including stillbirths.
No is the number of corpora lutea in third pregnancy.
N_A is the number of live fetuses in third pregnancy.
$N_D = N_O - N_A$ is the total mortality and includes ova not fertilized, as well as embryos which had died before the sow was slaughtered.
Percent mortality is $100N_D/N_O$.

suspect the stage of gestation to be more nearly alike for related than for non-related sows.

Sows were hand-fed a balanced ration of shelled corn and protein-mineral supplement at the rate of 6.0 lb. per day. This ration was less than the amount fed in previous breeding periods but was adequate to permit steady increase in weight of all sows.

For analysis, the data were classified in a hierarchal fashion. The sows sired by the same boar constituted a sire progeny, and several such progenies were in each breeding group.

The theoretical composition of mean squares and covariance is identical with that of Hazel *et al.*, (1943) and will not be repeated. In the present

TABLE 2. ANALYSIS OF VARIANCE FOR TRAITS SUMMARIZED IN TABLE 1

Source	Degrees of freedom	N_1	N_2	No	N_A	N_D	Percent mortality
		Mean squares					
Between breeding groups	4	49.48	70.98	19.23	4.12	16.03	300.5
Between sires within breeding groups	19	3.76	11.41	11.42	13.46	22.71	628.4
Between litters by the same sire	35	7.44	10.65	10.52	12.84	14.57	464.6
Between litter mates	28	5.70	9.58	6.40	9.32	16.08	409.5
		Components of variance [a]					
Between sires, V(S)		−1.07	0.21	0.23	0.15	2.36	46.8
Between litters by the same sire, V(M)		1.20	0.74	2.85	2.44	−1.04	38.1
Between litter mates, V(P)		5.70	9.58	6.40	9.32	16.08	409.5
V(S)+V(M)+V(P)		5.83	10.53	9.48	11.91	17.40	494.4

[a] The components of variance come from the equations:
"Between sires within breeding groups" mean squares = V(P)+1.482V(M)+3.469V(S).
"Between litters by the same sire" mean squares = V(P)+1.445V(M).
"Between litter mates" mean squares = V(P).

paper, the additive effects of all genes (G), of common litter environment (L) and of residual environment peculiar to individuals (E) were estimated as follows: $V(G)=4V(S)$, $V(L)=V(M)-V(S)$, and $V(E)=V(P)-2V(S)$ where $V(S)$ is the component of variance for sires, $V(M)$ is the component of variance for dams and $V(P)$ is the variance within litters.

Results and Discussion

The number of corpora lutea (N_O) averaged 16.4 (table 1) and ranged from 10 to 25. The distribution appeared nearly normal by visual inspection. The standard deviation within breeding groups was 3.1. The mean square (table 2) "between breeding groups" (19.23) is larger than the mean square between sires (11.42), although not significantly so on this volume of data. It suggests, but does not establish completely, that the breeding groups differ in number of ova shed.

The average sizes of the first (N_1), second (N_2) and slaughter (N_A) litters were 9.4, 11.2 and 9.7, respectively. Differences between breeding groups are significant at $P<.01$ for both first and second litters, but nonsignificant for the litter found at slaughter. Litter size increased from first to second litter but decreased from second to slaughter litter for all groups except the Poland Chinas. Increased litter size for the latter group appears largely responsible for failure to obtain differences between breeding groups at slaughter. Possibly maximum reproductive performance may be reached at an older age by the purebred Poland China sows than by sows of the various crossbred groups. Why the crossbred sows produced smaller litters at slaughter than in the previous litter is not clear. Performance in the previous reproduction or lactation period or both may have conditioned subsequent performance. The existence of a negative environmental correlation (that due to environment peculiar to individuals) (table 4) between second and slaughter litter size is consistent with the latter hypothesis.

Forty-one percent of the corpora lutea failed to produce live fetuses. This agrees closely with Casida's conclusion. Poland China sows had the lowest percent mortality but differences between groups were not significant.

Dead fetuses equalled 4.8% of the corpora lutea. Heterogeneity of the ratio of dead fetuses to number of corpora lutea was tested by chi-square on the same hierarchal classification employed for the analysis of variance. The results appear in table 3. Heterogeneity is indicated between sires of the same breeding group and approached significance for different litters by the same sire. Heterogeneity of differences between breeding groups and between litter mates does not appear. Forty-two sows that contained dead fetuses averaged 16.5 corpora lutea, 10.3 live fetuses and 38% mortality. Forty-five sows containing no dead fetus averaged 16.4 corpora lutea, 9.6 live fetuses and 44% mortality. Litters characterized by low

early mortality tended to have high late mortality. Heterogeneity between sires seems to be evidence that inheritance was a contributing factor.

Heritability

Estimates of heritability were not dependable, owing to the small volume of data, but a brief presentation is justified because very little evidence is available elsewhere.

Heritability of corpora lutea count is 10% when computed by the paternal half-sib method, using the components in table 2. The maternal component of variance is larger than the sire component of variance. This suggests that common environment of full sisters was important or that dominance or epistasis or both contributed considerably to the genetic

TABLE 3. CHI-SQUARE TESTS OF HETEROGENEITY OF THE RATIO OF DEAD FETUSES TO NUMBER OF CORPORA LUTEA

Source	Degrees freedom	Chi-square	Probability
Between breeding groups	5	5.31	.5–.3
Between boars of the same breeding group	19	32.85	.05–.02
Between litters by the same boar	35	41.89	.10–.05
Between litter mates	28	28.04	.5–.3

variation. Squiers *et al.*, (1952) also found suggestions that maternal environment was important.

Heritability of litter size, computed by the paternal half-sib method, is 8% for the second litter and 5% for the third litter. Heritability for gilt litter size is assumed to be zero because the variance between paternal half-sisters is less than that between full-sisters. Repeatabilities are .15 between first and second litters, .10 between second and third litters and —.06 between first and third litters.

The maternal component of variance is negative for total mortality ($N_D=N_O-N_A$). If this component is assumed to be zero, heritability is 21% when computed by the full-sib correlation method. Heritability of percent mortality ($100N_D/N_O$) is 38% when computed by the paternal half-sib method.

Genetic and Environmental Correlations

Genetic and environmental correlations are summarized in table 4. This tabulation is incomplete because negative components of variance made some correlations indeterminate. Large sampling errors are reflected by several values larger than one or less than minus one.

The genetic correlation between number of corpora lutea and size of

second litter is negative as is that between number of corpora lutea and size of third litter. This suggests that additively genetic factors which caused increased ovulation also caused decreased litter size. Environmental factors common to litter mates and affecting number of corpora lutea are positively correlated with those affecting size of third litter. The residual correlation (that due to environment peculiar to individuals) is also positive, suggesting that individual sow environment which favored increased ovulation also favored increased litter size. The residual correlation between size of second and third litters is negative as it would be if greater stress in a previous pregnancy caused poorer response in the next. It appears that environmental factors, possibly associated with reproduc-

TABLE 4. GENETIC, PHENOTYPIC AND ENVIRONMENTAL CORRELATIONS WITHIN BREEDING GROUPS [a]

Source		Trait N_1	N_2	N_O	N_A	N_D		Source
		....	....	....	....		G_1	
Phenotypic				−1.91	3.83	−1.56	G_2	Genetic
correlation	N_2	.15			−5.35	1.65	G_O	correlation
	N_O	.18	.22			−1.92	G_A	
	N_A	−.06	.10	.19				
	N_D	.18	.08	.58	−.69			
			.16	.73	.42	.16	E_1	Correlation due
				.54	−.30	.66	E_2	to environment
Correlation due	L_2	.35			.24	.50	E_O	peculiar to
to environment	L_O	.90	−.08			−.71	E_A	individuals
common to	L_A	.20	1.02	1.70				
litter mates	L_D	....	....	....	....			

[a] This table is incomplete because negative components of variance made some correlations indeterminate.

tion or lactation of the previous pregnancy, play important roles in conditioning reproductive performance. This is in general agreement with the work of Self *et al.*, (1955) demonstrating the effect of feeding level on ovulation and embryonic survival. However, the positive environmental correlation between number of corpora lutea and number of live fetuses appears to disagree with their results.

The phenotypic correlations between number of corpora lutea and size of litter are .18, .22 and .19 for first, second and third litters, respectively. Corpora lutea of the third pregnancy predicted litter size of that litter and of earlier litters equally well.

Summary

Ovulation, prenatal mortality and litter size were observed for 87 sows, representing five breeding groups, that had previously farrowed two litters. Heritabilities and correlations arising from additively genetic sources, common litter environment and residual environment were computed.

The number of live fetuses was 59% of the number of corpora lutea and dead fetuses accounted for 5%.

Repeatability of litter size was .15 between first and second litters, .10 between second and third litters and —.06 between first and third litters. Heritability of litter size was 0, 8 and 5% for first, second and third litters, respectively. Heritability of corpora lutea count was 10%. Genetic and environmental correlations are summarized. None of the genetic parameters are statistically significant.

Literature Cited

Casida, L. E. 1952. Fertilization failure and embryonic death in domestic animals. In Engle, E. T., ed. Pregnancy Wastage. p. 27–37, Springfield, Ill., Charles C Thomas, publisher.

Corner, G. W. 1923. The problem of embryonic pathology in mammals with observations upon intrauterine mortality in the pig. Am. J. Anat. 31:523.

Hammond, J. 1914. On some factors controlling fertility in domestic animals. J. Agr. Sci. 6:263.

Hammond, J. 1921. Further observations on the factors controlling fertility and fetal atrophy. J. Agr. Sci. 11:337–366.

Hazel, L. N., M. L. Baker and C. F. Reinmiller. 1943. Genetic and environmental correlations between the growth rates of pigs at different ages. J. Animal Sci. 2:118.

Robertson, G. L., R. H. Grummer, L. E. Casida and A. B. Chapman. 1951. Age at puberty and related phenomena in outbred Chester White and Poland China gilts. J. Animal Sci. 10:647.

Self, H. L., R. H. Grummer and L. E. Casida. 1955. The effects of various sequences of full and limited feeding on the reproductive phenomena in Chester White and Poland China gilts. J. Animal Sci. 14:573.

Squiers, C. D., G. E. Dickerson and D. T. Mayer. 1952. Influence of inbreeding, age and growth rate of sows on sexual maturity, rate of ovulation, fertilization and embryonic survival. Mo. Agr. Exp. Sta. Res. Bul. 494.

THE INCIDENCE OF EXTRAMEDULLARY MYELOPOIESIS IN BOVINE ADRENAL GLANDS [1]

J. S. GREENSTEIN AND R. C. FOLEY
University of Massachusetts [2]

INVESTIGATORS currently studying the anatomy, physiology, and biochemistry of the bovine adrenal gland are attempting to clarify the functional significance of this important endocrine organ in relation to production, reproduction, metabolism, and growth. The recognition and exploration of adrenal disorders contribute in large measure to the final understanding of its endocrine functions.

In a study of bovine adrenal glands from fetuses, calves, heifers, and cows, Weber *et al.* (1950) noted the occurrence of small areas of extramedullary myelopoiesis in approximately one of every three adult glands studied. The hemopoietic areas obliterated the cortical cells throughout the width of the cortex and often involved the capsule. The affected areas generally assumed a cone-shape with the base situated in the glomerulosa region.

Cupps *et al.* (1953) studied left adrenal glands from three groups of cattle and reported the incidence of myelopoietic areas in pregnant Herefords, in sexually mature virgin Hereford heifers, and in Jerseys 2 to 10 years of age. The hemopoietic areas were found in all the zones of the cortex occurring most often in the inner zone of the cortex with the base of the cone in the reticularis. The physiological significance of these areas was not determined, but the authors suggested that the marked difference in the incidence of myelopoiesis in the different groups of animals studied could be related to the breed of cattle, age, or sire family. The relationship between infertility and high incidence of extramedullary myelopoiesis was considered problematical.

The stages in the development of extramedullary myelopoiesis have been described by Maximow and Bloom (1952), and the histogenesis and morphology of benign myeloproliferation have been reviewed by Peace (1953). The myeloproliferative syndromes are considered by Peace to be nonspecific responses by the primitive mesenchymal cell system to various types of injury. Although etiologic agents including toxic chemicals, toxemias of systemic inflammatory diseases, liver dysfunction, and endocrine disorders have been implicated, the causative factor is frequently unknown.

The following study of the incidence of hematopoiesis in the adrenal glands of cattle of six dairy and beef breeds is an outgrowth of a larger

[1] Supported by the Northeast Cooperative regional project NE-1 "Causes and Prevention of Reproductive Failures in Cattle" and by funds from Dairy Husbandry Research Branch, USDA, Beltsville, Maryland.
[2] Cont. No. 1075 of the University of Massachusetts, College of Agriculture Experiment Station, Amherst.

investigation concerned with the causes and prevention of reproductive failures in cattle. A total of 105 head of cattle has been slaughtered in this project. Detailed information was available relative to age, stage of gestation, ancestry, and previous breeding history of all individuals.

Materials and Methods

The cattle slaughtered in the experiment were culled from the University of Massachusetts dairy and beef herds for inferior type, low production, poor family, mastitis, low fertility or for combinations of these factors.

The group included 69 cows and 36 heifers, of which 81 were pregnant and 24 were open at the time of slaughter. The distribution by breeds was as follows: Aberdeen-Angus 12, Ayrshire 26, Guernsey 25, Hereford 4, Holstein 24, and Jersey 14.

Representative portions of both left and right adrenal glands were fixed in several fixatives including Bouin's, Rossman's, formol-sublimate, and acetic-alcohol formalin. Tissues embedded in Tissuemat were sectioned at 6 micra and stained with each of the following four stains: modified Mallory, hematoxylin and eosin, azure-eosin, and modified phloxine-methylene blue.

Results

Foci of myeloproliferation which obliterated the normal cortical structure were found in all the zones of the cortex. The sizes, shapes, and locations of the myelopoietic areas varied considerably from gland to gland, ranging from a limited area in a single zone (figure 1) to broad areas involving the glomerulosa, fasciculata, and reticularis (figure 3). For comparison, corresponding areas of normal adrenal cortical tissue are shown in figure 4.

The areas involved were usually cone-shaped with a broad base lying either in the reticularis (figure 5) or in the glomerulosa, the former being more common. However, the shape of the area is largely a function of the plane of the particular section, and the total extent of the involvement in any gland can be assessed only by serially sectioning the entire gland. In figure 2, myeloproliferation is confined largely to the zona fasciculata but extends also into the reticularis.

The areas of myeloproliferation were composed of cells of the myeloid series (figure 7). The predominant type was identified as eosinophil myelocytes, but other immature granulocytes as well as neutrophils, eosinophils, and megakaryocytes were in evidence. In rare instances, the myeloid cells extended into the medulla and were observed in the medullary capillaries (figure 8).

Areas of hematopoiesis were identified in 24 of 105 animals, an over-all incidence of 22.9%. In an effort to explain the significance of the hematopoietic areas, the data were organized according to breed, age, sires, pregnancy, and the previous reproductive histories of the cows and heifers.

Incidence by Breeds. The incidence of foci of extramedullary hematopoie-

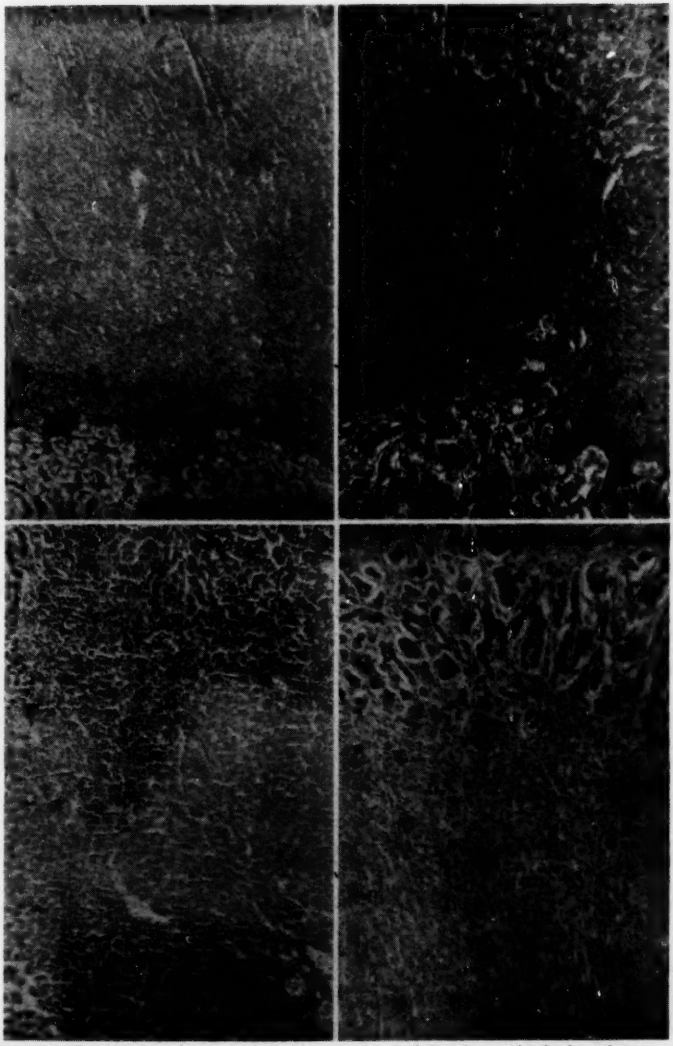

Figure 1. Broad focal area of hemopoietic activity in reticularis adjacent to medulla. Azure-eosin. X75.

Figure 2. Area of myeloproliferation largely confined to zona fasciculata but extending into reticularis. Modified Mallory. X75.

Figure 3. Extramedullary myelopoiesis involving all three zones of the cortex. Azure-eosin. X75.

Figure 4. Normal adrenal cortex showing capsule, glomerulosa, and inner zones. Azure-eosin. X75.

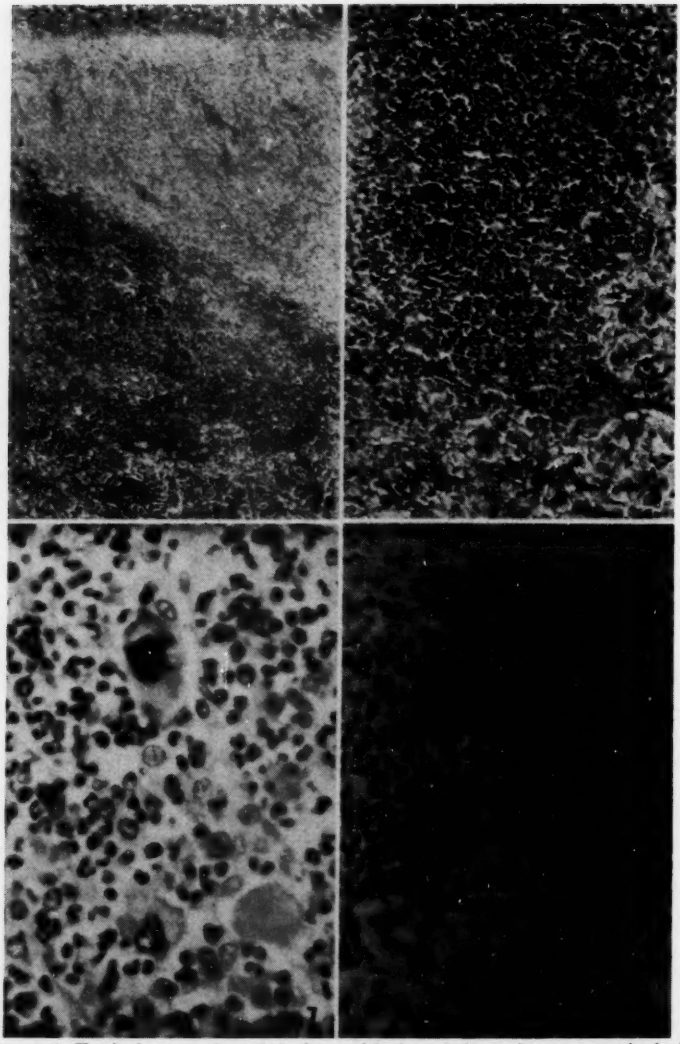

Figure 5. Typical cone-shaped lesion with broad base in zona reticularis. Phloxine-methylene blue. X31.

Figure 6. Dense leucocytic area with several prominent megakaryocytes. Pholxine-methylene blue. X125.

Figure 7. High power view of cell types in hematopoietic area. Hematoxylin-eosin. X600.

Figure 8. Proliferating granulocytes in zona reticularis and extending into medullary capillaries. Azure eosin. X300.

TABLE 1. INCIDENCE OF ADRENAL HEMATOPOIESIS BY BREEDS

Breed	No. of cattle	Hematopoiesis		Incidence
		Present	Absent	
				%
Aberdeen-Angus	12	5	7	41.7
Ayrshire	26	3	23	11.5
Guernsey	25	5	20	20.0
Hereford	4	0	4	0.0
Holstein	24	4	20	16.0
Jersey	14	7	7	50.0
Totals	105	24	81	22.9

sis in the various breeds is indicated in table 1. The highest incidence (50%) occurred in the Jersey breed followed in descending order by the Aberdeen-Angus, Guernsey, Holstein, and Ayrshire. In the four Herefords studied, no evidence of the condition was found.

Incidence by Age Groups. The 105 head of cattle ranged in age from 15 months to 11 years and 7 months. Thirty-six were heifers between one and two years of age, which had never calved; 69 were cows that had had one or more calves. The distribution of the animals by age groups is listed in table 2. There appears to be an increased incidence of hematopoiesis among the cows with advancing age.

Incidence by Sires. Forty-four sires were represented among the 105 daughters. Of these, 15 sires or 34.1% had at least one daughter exhibiting extramedullary myelopoiesis. In view of the fact that 25 of the sires in this study had only one daughter each, the percentage of sires with positive daughters in regard to this condition might have been higher if additional daughters had been available for sampling. Among the 47 daughters of the 15 sires with at least one positive daughter, 51% were affected.

With regard to the total incidence reported for a given breed as indicated in table 1, the degree of influence exerted by individual sires varied widely.

TABLE 2. INCIDENCE BY AGE GROUPS

	No. of cattle	Hematopoiesis		Incidence
		Present	Absent	
				%
Heifers 1–2 years	36	6	30	16.7
Cows 2–3 years	9	1	8	11.1
Cows 4–5 years	25	6	19	24.0
Cows 6–7 years	16	5	11	31.3
Cows 8 or over	19	6	13	31.6
Totals	105	24	81	22.9

TABLE 3. INCIDENCE IN RELATION TO PREGNANCY

	No. of cattle	No. positive	No. negative	Percentage positive
Open cattle	24	8	16	33.3
Pregnant cattle	81	16	65	19.8

For example, four of the five positive cattle in the Angus breed were daughters of a single sire, whereas in the Jersey breed five different sires were represented among the seven positive daughters. Similarly, the incidence among the daughters of an individual sire ranged from 15.4% for the progeny of an Ayrshire bull with two of 13 positive daughters to 100% among the progeny of a Jersey bull with three of three positive daughters.

Incidence Relative to Pregnancy. As indicated in table 3, this condition apparently was not related to pregnancy, occurring in 33% of the open cows and heifers and in 20% of the pregnant animals.

Incidence in Relation to Breeding History. In evaluating any possible relationship between the incidence of hematopoiesis and fertility, the cattle were divided into three groups based upon the breeding health, previous history, number of services required for conception, and post mortem findings in the reproductive tracts. The 55 cattle in Group I were normal in breeding health, conceived on the first or second service, and yielded normal embryos at the time of slaughter. The 30 head in Group II had a history of breeding troubles, were abnormal upon post-mortem examination, or required from 5 to 10 services for conception. Those cattle that did not clearly belong in either Group I or Group II were classified as Group III, or doubtful animals. The incidence by groups is shown in table 4.

The 13 cases among the normal cattle, as well as the fact that the incidence in the abnormal group is almost exactly the same as in Group I, would suggest that whatever the explanation for this condition, it is not closely related to the level of fertility in cattle.

Discussion

The relatively high incidence of myelopoietic areas in the bovine adrenal glands reported in this paper is in agreement with the previous reports of

TABLE 4. INCIDENCE IN RELATION TO BREEDING HISTORY

	Group	Total No. of cattle	Number positive	Number negative	Incidence by group
					%
Normal cattle	I	55	13	42	23.6
Abnormal cattle	II	30	7	23	23.3
Doubtful cattle	III	20	4	16	20.0
Totals		105	24	81	22.9

Weber *et al.* (1950) and Cupps *et al.* (1953). The incidence may be higher than these data indicate, since in no case have the glands been examined in entirety. There is apparently no parallel for this high incidence recorded for any other species in the literature of the myeloproliferative syndromes. The occurrence of extramedullary myelopoiesis in humans is regarded as a disease, which is generally accompanied by a variety of recognized clinical manifestations. All the evidence to date indicates that the condition in the bovine does not bear any relation to known disease factors.

The incidence of 50% among 14 Jersey cows and heifers slaughtered in this investigation is comparable to the figure of 57.1% reported by Cupps *et al.* (1953) for a group of 35 Jerseys. In the latter instance, 23 of the animals in the Jersey group were the progeny of one sire, and 16 of the 23 had leucocytic areas in the gland. In the present Jersey group, 11 sires are represented in the 14 animals and the three progeny of one sire all had leucocytic areas, whereas the remaining four cases were distributed evenly among four other sires.

The situation among the Aberdeen-Angus where incidence was also especially high was as follows: four sires were represented in the 12 animals, and of the five animals showing leucocytic areas, four had a common sire, whereas two other animals from the same sire were negative in this regard.

It is worthy of note that Cupps *et al.* reported no myelopoietic areas in glands of 12 pregnant Herefords studied. Similarly the four negative Herefords in the present study were all pregnant.

The trend toward an increased incidence with advancing age, as indicated in table 2, is suggestive in view of the results reported by Cupps *et al.* on a similar age basis. The incidence of myeloid areas for 35 Jerseys was reported by them as follows: 2 and 3 years, 16.6%; 4 and 5 years, 69%; 6 and 7 years, 80%; and 8 or older, 33.3%.

While it is not possible to evaluate the influence of the sire on the frequency of the condition in the total cattle population based on the presently available information, the wide variation in incidence among breeds and the high incidence among the progeny of sires with one or more positive daughters suggest a possible genetic relationship. Such a relationship, of course, cannot be established without access to more extensive information on whole family groups.

As indicated in tables 3 and 4, the condition does not appear to be related to pregnancy or reproductive performance. The latter inference is not in agreement with a recent publication of Cupps *et al.* (1956), which indicated a high incidence among an apparently select group of dairy cows exhibiting impaired reproductive performance.

The physiological significance of these lesions in the bovine adrenal glands remains unknown. The possible relationship between the condition and productive performance of cattle has not been explored. The relatively high incidence of the condition among this heterogeneous group of culls

would indicate that further investigation is warranted in the interests of maximum production and utilization of our livestock resources.

Summary

Extramedullary myelopoiesis appears to be a relatively common condition in the adrenal glands of cull dairy and beef cattle, occurring in 23% of 105 animals examined. The lesions involve any or all of the zones of the cortex and vary substantially in shape and size from gland to gland.

The data from this investigation, based upon limited numbers, indicate that the condition is not related to fertility or to pregnancy. The breed and sire data in this investigation suggest a possible genetic basis but this cannot be demonstrated without further investigation. The syndrome may be associated with the age of the individuals, being more common in older cattle.

Literature Cited

Cupps, P. T., R. C. Laben and S. W. Mead. 1953. The occurrence of extramedullary myelopoiesis in the adrenal cortex of the cow. Cornell Vet. 43:244.

Cupps, P. T., R. C. Laben and S. W. Mead. 1956. Histology of the pituitaries, adrenals, ovaries, and uteri of dairy cattle associated with different reproductive conditions. J. Dairy Sci. 39:155.

Maximow, A. E. and W. Bloom. 1952. A Textbook of Histology. 6th Edition, W. B. Saunders Co., Philadelphia.

Peace, R. F. 1953. Myelonecrosis, extramedullary myelopoiesis, and leuko-erythroblastosis. A mesenchymal reaction to injury. Amer. J. Path. 29:1029.

Weber, A. F., S. H. McNutt and B. B. Morgan. 1950. Structure and arrangement of zona glomerulosa cells in the bovine adrenal. J. Morph. 87:393.

THE RELATIVE EMPHASIS PLACED ON COMPONENTS OF A SELECTION INDEX UNDER TWO NUTRITIONAL ENVIRONMENTS[1, 2, 3]

R. E. CHRISTIAN

State College of Washington

THEORETICALLY, the most effective method of selection among farm animals is through the use of a selection index (Hazel and Lush, 1942). However, most selection indexes have been developed for use in a particular herd or group of herds under more or less constant environment. Bernard *et al.* (1954) proposed a selection index for swine to be used under farm conditions. It is generally assumed that an index developed for use under one environment may not be effective when used under other conditions. The present study was undertaken to determine the relative emphasis placed on traits in a selection index when used in the same herd under two different nutritional environments.

Material and Methods

The data in this study are taken from the first six generations of swine on an experiment designed to determine the relative amount of progress possible when selecting under optimum vs. limited nutrition. The foundation stock for this project was created by crossing five Danish Landrace boars with five unrelated groups of five Chester White sows each. The resulting offspring from each group were divided, at weaning time, into two lines: one line (called high-plane, H) was full-fed from weaning to 150 lb., the other line (called low-plane, L) was fed 70% as much as the high-plane pigs ate at comparable weights up to 150 lb. From 150 lb. in weight until farrowing all pigs were hand-fed to prevent the high-plane pigs from becoming obese. The high-plane animals received sufficient feed to allow them to gain approximately 1.5–2.0 lb. daily; the low-plane pigs received 70% of this amount. Following farrowing the high-plane sows were again full-fed and the low-plane sows received 70% of full feed. The same rations were fed to both high- and low-plane pigs (table 1). All animals were maintained in dry lot throughout the study. The rations were

[1] Scientific Paper No. 1511, Washington Agricultural Experiment Station, Pullman. Project No. 807.

[2] In cooperation with Swift and Co., Chicago, Ill., with added support from Carstens Packing Co., Tacoma and Spokane, Wash.; Gibson Packing Co., Yakima, Wash.; American Dry Milk Institute, Inc., Chicago, Ill.; Hiram Walker, Peoria, Ill.; Standard Brands, Inc., New York, N. Y.; Charles Pfizer and Co., Brooklyn, N. Y.

[3] The advisory committee for this project is as follows: S. P. Swenson, Dean, College of Agriculture, State College of Washington, Pullman, Wash.; H. E. Robinson, Director of Research, Swift and Co., Chicago, Ill.; E. J. Warwick, Cattle Research Center, USDA, Beltsville, Md.; A. J. Wood, Department of Animal Husbandry, University of British Columbia, Vancouver, B. C., Canada; C. F. Sierk, Office of Food and Agriculture, ICA, Washington, D. C.; Ray Meenach, President, Washington Swine Breeders Ass'n., Valleyford, Wash.; M. E. Ensminger, Department of Animal Husbandry, State College of Washington, Pullman, Wash.

designed to provide adequate protein, minerals and vitamins at the levels fed to low-plane pigs, the only deficiency being energy. Thus the high-plane pigs received adequate energy but an excess of protein, minerals and vitamins.

Breeding stock was selected as the pigs reached 150 lb. on the basis of the following index: $I=B+2W+35G$, where B is the number of pigs born alive and W is the number of pigs weaned in the litter in which the individual pig was born, and G is the average rate of daily gain of the individual pig from weaning to 150 lb. This index was designed to place approximately equal emphasis on prolificacy and rate of gain based on variability. No attention was given to desirability of type.

TABLE 1. RATIONS FED

Ingredients	Weaning to 75 lb.	76 lb. to 150 lb. and lactating sows	Pigs over 150 lb. and gestating sows
	lb.	lb.	lb.
Ground barley	32.0	30.0	31.0
Ground wheat	22.0	30.0	24.0
Mill run	0.8	0.7	0.7
Corn distillers dried solubles	0.7	0.6	0.6
Soybean meal	17.0	14.0	14.0
Fish meal	11.0	9.0	9.0
Dried skim milk	5.0	4.0	4.0
Dehydrated alfalfa meal	9.5	10.5	15.0
Ground oyster shell	0.7	0.6	0.6
Steamed bone meal	0.7	0.6	0.6
Iodized salt	0.6	0.5	0.5
Mineral Mix [a]	0.08	0.08	0.08

[a] The mineral mix was made up of the following: $ZnCO_3$, 50 gm.; KI, 50 gm.; $CuSO_4$, 150 gm.; Fe_2O_3, 200 gm.; $FeSO_4$, 5000 gm. $MnSO_4$, 200 gm.; plus irradiated yeast, type 9F, 1820 gm., and soybean oil meal, 2500 gm.

These two lines were maintained as closed populations throughout. Inbreeding was kept at a minimum by systematic crossing of the groups within lines.

From each of the five groups within each line, one boar and three gilts were selected for replacement breeding stock making a total of 5 boars and 15 gilts saved in each plane each generation. In the third generation one group was lost in H and the comparable group in L was eliminated leaving 4 groups in each plane.

All litters were from gilts and were born within a two month interval each year.

Two methods were used to determine the emphasis placed on each of the traits in the selection index: The first was the method of path coefficients (Wright, 1934). Using the path diagram shown in figure 1 in which index (I) is completely determined (at least mathematically) by number

born (B), number weaned (W), and average daily gain (G), the following four simultaneous equations result:

$$r_{IB}=b+wr_{BW}+gr_{BG} \quad (1)$$
$$r_{IW}=w+br_{BW}+gr_{WG} \quad (2)$$
$$r_{IG}=g+br_{BG}+wr_{WG} \quad (3)$$
$$1=b^2+w^2+g^2+2br_{BW}w+2wr_{WGG}+2br_{BGG} \quad (4)$$

[4] r=simple correlation.
b, w, g=path coefficients from index to No. born, No. weaned and average daily gain, respectively.

Within-sex, within-plane correlations were used in calculating the values of the various paths, and the values for the two sexes were averaged. The squared path coefficient represents the proportion of the variance in the dependent variable for which the independent variable is directly responsible.

The other method used was to compare the intensity of selection for each of the traits used in the selection index. This was accomplished by dividing

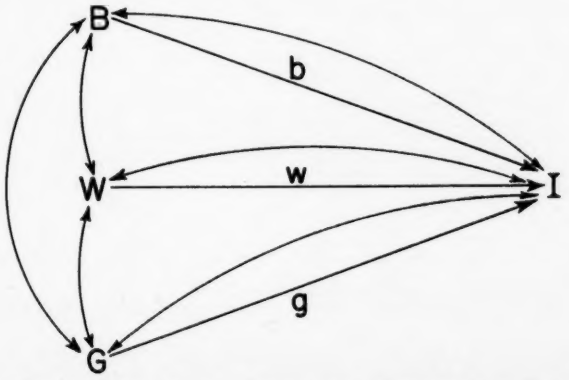

Figure 1. Path coefficient diagram showing the relationship between index (I) and number born (B), number weaned (W) and average daily gain (G).

the weighted selection differentials by their respective standard deviations. In this form they can be compared directly.

Results and Discussion

As shown in table 2 the average number of pigs born alive per litter was 8.85 for H and 9.26 for L. This difference of 0.41 in favor of L was not significant statistically. Neither does there appear to have been any increase in number of pigs born alive per litter with 5 generations of selection. The average number of pigs weaned per litter was 5.65 for H and 6.63 for L. This difference of 0.98 pig in favor of L was highly significant

statistically. As with number born alive per litter, it appears that five generations of selection was ineffective in increasing average litter size weaned.

This is in agreement with the results of Dickerson *et al.* (1954). They reported that selection for litter size in inbred lines has been rather ineffective at several experiment stations cooperating in the Regional Swine Breeding Laboratory.

The average weight per pig at weaning was 36.5 lb. in H and 28.5 lb. in L. This highly significant difference of 8.0 lb. in favor of H might be expected since the feed of L sows was limited to approximately 70% of the consumption of H sows. This undoubtedly resulted in a lowered milk

TABLE 2. PERFORMANCE OF FIRST SIX GENERATIONS OF PIGS RAISED ON FULL FEED (HIGH PLANE) AND 70% OF FULL FEED (LOW PLANE)

	Av. no. pigs born per litter		Av. no. pigs weaned per litter		Av. weaning weight (lb.)		Av. daily gain from weaning to 150 lb.	
	Plane of nutrition							
Generation	High	Low	High	Low	High	Low	High	Low
1	9.26 [a]		7.79		27.1		1.21	0.77
2	10.05	10.31	6.16	7.00	30.0	22.1	1.24	0.78
3	7.71	8.91	3.59	5.18	37.1	34.0	1.45	0.80
4	10.07	9.00	7.85	7.05	43.2	31.7	1.44	0.98
5	6.60	8.82	3.67	6.47	40.3	28.2	1.48	1.02
6	9.82	9.25	7.00	7.44	31.7	26.7	1.06	0.99
Unweighted mean [b]	8.85	9.26	5.65	6.63	36.5	28.5	1.31	0.89
Difference (H—L)	—0.41		—0.98**		8.0**		0.42**	

** $P<.01$.

[a] Pigs were assigned to their respective planes at weaning time.

[b] Unweighted mean for No. born, No. weaned and weaning weight based on generations 2–6.

production by L sows. Also, since the H sows were self-fed, the young pigs had access to feed and although they consumed relatively little they were in a sense creep-fed.

The average rate of daily gain from weaning to 150 lb. was 1.31 lb. for H pigs and 0.89 lb. for L pigs. This highly significant difference of 0.42 lb. in favor of H pigs would be expected since they were self-fed while the L pigs were limited to 70% of full feed. With the exception of the sixth generation, it would appear that selection for rate of gain was rather effective in both planes. The H pigs increased steadily from an average of 1.21 lb. in the first generation to 1.48 lb. in the fifth generation, an increase of 0.27 lb. The L pigs increased from 0.77 lb. in the first generation to 1.02 lb. in the fifth generation, an increase of 0.25 lb. One explanation for the lowered performance in the sixth generation is that there was an outbreak of hemorrhagic enteritis after weaning in both planes, which appeared to be more severe in the H pigs.

The values of the squared path coefficients for the six generations are given in table 3. The percentage of the variance in index for which number born was directly responsible (b^2) varied from 6 to 18 in the high plane and 9 to 21 in the low plane, the averages being 10.5% and 14.6% respectively, a difference of only 4.1%.

Number weaned accounted for from 18 to 42% of the variance in index in H and from 34 to 76% in L. The average was 32.7% in H and 50.6% in L, a difference of 17.9%.

Rate of gain accounted for from 22 to 74% of the variance in index in H and 26 to 58% in L. The average was 40.1% in H and 39.4% in L, a

TABLE 3. SQUARED PATH COEFFICIENTS TO INDEX FROM NO. BORN (b), NO. WEANED (w), AND AVERAGE DAILY GAIN (g)

Plane of nutrition	Generation	b^2	w^2	$b^2 + w^2$	g^2
High	1	.0566	.1806	.2372	.5329
	2	.1875	.4199	.6074	.5595
	3	.1050	.3982	.5032	.2959
	4	.0807	.1823	.2630	.7430
	5	.1274	.3446	.4720	.2621
	6	.0858	.4122	.4980	.2162
	Av.[a]	.1050	.3272	.4322	.4007
Low	1	.0900	.3422	.4322	.2756
	2	.1030	.5491	.6521	.2798
	3	.1282	.4058	.5340	.2611
	4	.2134	.3906	.6040	.4942
	5	.2088	.5565	.7653	.5329
	6	.1246	.7586	.8832	.5837
	Av.	.1459	.5055	.6514	.3944

[a] Based on within-sex, within-generation correlations.

difference of only 0.7%. When the squared path coefficients to index from number born and from number weaned are added together this gives an estimate of the percentage of the variance in index which is accounted for by "prolificacy". In H, b^2+w^2 varied from .24 to .61, and in L from .43 to .88, the average being .432 in H and .651 in L, a difference of .219.

One possible explanation of this difference is that the squared path coefficients represent only the direct influence of these two factors on the index, while the terms for joint determination in equation (4) have not been considered since there is no way to partition them. Also, there are larger negative correlations between B and G in L than in H.

As stated in the introduction, the index was designed to place equal emphasis on prolificacy and rate of gain. Table 3 shows that this was approximately true in H (.432 vs. .401) but in L there was more emphasis on prolificacy (.651) than on gain (.394). This would indicate that the selection index used in this study was not designed for use with data from

animals on limited feed. It is likely that this difference is due to the higher negative correlation between W and G in L (—.287) than in H (—.015). This difference is significant statistically ($P<.01$). This larger correlation may have arisen because the feed of L sows was limited during lactation. This in turn limited the milk supply to individual pigs in large litters and resulted in smaller pigs at weaning time. These pigs then received less feed after weaning since the amount of feed fed to L pigs was based on their own body weight. Therefore, the lighter pigs had relatively less feed for growth after weaning than heavier pigs and consequently grew slower. The same situation did not occur in H pigs since the H sows were full-fed and the H pigs were full-fed after weaning.

TABLE 4. INTENSITY OF SELECTION FOR NO. BORN, NO. WEANED, AV. DAILY GAIN, AND INDEX

Plane of nutrition	Generation	No. born		No. weaned		Av. daily gain		Index	
		S. D.[a]	S. D. s[b]	S. D.	S. D. s	S. D.	S. D. s	S. D.	S. D. s
High	1	0.238	0.150	0.547	0.409	0.118	0.811	5.452	0.778
	2	0.774	0.401	0.129	0.092	0.068	0.691	3.421	0.750
	3	—0.333	—0.157	—0.436	—0.182	0.127	1.121	2.150	0.281
	4	0.216	0.166	0.650	0.494	0.032	0.215	2.656	0.448
	5	0.460	0.151	0.506	0.194	0.078	0.556	4.194	0.481
	6	0.960	0.490	1.103	0.506	0.042	0.420	4.612	0.670
	Unw. Av.	0.386	0.200	0.416	0.252	0.078	0.636	3.748	0.568
Low	1	0.794	0.526	0.911	0.622	0.044	0.551	4.155	0.828
	2	0.776	0.409	1.098	0.458	0.024	0.266	3.948	0.653
	3	0.564	0.303	0.416	0.248	0.079	0.882	4.166	0.760
	4	0.907	0.414	1.120	0.757	0.042	0.433	4.594	0.966
	5	0.928	0.398	1.270	0.670	0.075	0.698	6.096	1.216
	6	0.685	0.522	0.986	0.615	0.049	0.560	4.380	1.175
	Unw. Av.	0.776	0.429	0.967	0.562	0.052	0.565	4.556	0.933

[a] Weighted selection differential.
[b] Standard deviation.

The actual amount of selection practiced per generation for the three traits included in the selection index is shown in table 4. There was positive selection for all three traits in all generations except for B and W in generation 3, high plane. The selection differentials as reported for B and W are for deliberate selection and do not include automatic selection for litter size (see Dickerson *et al.*, 1954). If the automatic selection is included the unweighted average selection differentials become H: B, 1.20; W, 1.73; and L: B, 1.25; W, 2.17.

Selection differentials expressed in different units become comparable when divided by their respective standard deviations. When this is done it is apparent that the selection for B and W was approximately twice as intense in L as in H, while the selection for G was not materially different in the two groups. Within H the selection for G exceeded the total selection

for B and W, while in L the total selection for B and W was almost twice the selection for G.

Also, the intensity of selection for index was greater in L than in H. This may have been due partly to more offspring being available from which to select in L than in H. In H an average of 46.6% of available females and 14.6% of available males were saved for breeding while in L the corresponding percentages were 40.3 and 11.6. According to Lush (1945), if 46.6% of the females and 14.6% of the males are saved for breeding the selection differential could be approximately 1.20 standard deviations, while if 40.3% of the females and 11.6% of the males are saved it could be approximately 1.32 standard deviations.

The fact that the two populations are not distributed normally may account for an additional portion, although neither population deviated significantly from a normal distribution.

Summary

The data from two lines of swine, descended from a common stock, that were maintained as closed populations for six generations were used in this study.

One line (H) was full-fed from weaning to 150 lb., hand-fed from 150 lb. to parturition at levels which permitted gains of 1.5–2.0 lb. daily and again full-fed during lactation. The other line (L) received 70% as much feed as the H group throughout. All selections were made as each pig reached 150 lb. using the following index: I=B+2W+35G, where B is the number farrowed alive and W is the number weaned in the litter in which the individual pig was born, and G is the average rate of daily gain of the individual pig from weaning to 150 lb.

There was no significant difference between H and L in average number of pigs farrowed alive per litter (8.85 vs. 9.26). However, there was a highly significant difference between H and L in average number of pigs weaned per litter (5.65 vs. 6.63), average weaning weight of the individual pig (36.5 lb. vs. 28.5 lb.) and average rate of daily gain from weaning to 150 lb. (1.31 lb. vs. 0.89 lb.).

Selection apparently was ineffective in increasing litter size in either line. However, selection for rate of daily gain appeared to be effective in both lines.

As determined by the method of path coefficients, approximately equal emphasis was placed on prolificacy (B+2W) as on average daily gain in H, while in L more emphasis was placed on prolificacy than on average daily gain.

Using weighted selection differentials expressed in terms of standard deviations, somewhat less selection was practiced for prolificacy than for average daily gain in H, while in L there was considerably more selection for prolificacy than for average daily gain.

Literature Cited

Bernard, C. S., A. B. Chapman and R. H. Grummer. 1954. Selection of pigs under farm conditions: Kind and amount practiced and a recommended selection index. J. Animal Sci. 13:389.

Dickerson, G. E., C. T. Blunn, A. B. Chapman, R. M. Kottman, J. L. Krider, E. J. Warwick and J. A. Whatley, Jr. 1954. Evalulation of selection in developing inbred lines of swine. Mo. Agr. Exp. Sta. Res. Bul. 551.

Hazel, L. N. and J. L. Lush. 1942. The efficiency of three methods of selection. J. Hered. 33:393.

Lush, J. L. 1945. Animal Breeding Plans. Third Ed. Iowa State College Press, Ames.

Wright, S. 1934. The method of path coefficients. Ann. Math. Stat. 5:161.

THE VALUE OF VARIOUS BLENDS OF SOYBEAN OIL MEAL PEANUT OIL MEAL, AND DEGOSSYPOLIZED COTTONSEED OIL MEAL AS PROTEIN SUPPLEMENTS IN SWINE RATIONS [1]

R. F. Sewell, B. C. Keen and J. L. Carmon
University of Georgia [2]

THE situation frequently exists where price differentials among the high protein oil meals, as employed in swine feeds and supplements, favor the use of cottonseed oil meal and peanut oil meal, particularly in the areas where these products are processed.

With the advent of low-gossypol cottonseed oil meals and the advances in our knowledge of amino acid requirements and non-protein dietary factors, there is an apparent need for more information concerning the use of these ingredients in swine rations.

Wallace *et al.* (1955) reported significantly faster gains with pigs receiving a combination of soybean oil meal and low-gossypol cottonseed oil meal as compared to a fortified diet containing soybean oil meal alone.

In chick experiments, Blaylock and Richardson (1950) obtained a growth response to L-lysine supplementation on diets containing a combination of soy bean oil meal and cottonseed oil meal. In the same study it was indicated that methionine was a limiting factor on a diet containing a combination of soybean oil meal and peanut oil meal.

The purpose of the studies reported here was to provide additional information on the nutritional value of soybean oil meal, "degossypolized" cottonseed oil meal and peanut oil meal when used alone and in various blends as protein supplements in rations for growing swine.

Experimental Procedures

Experiment I. Sixty weanling Duroc and Duroc X Yorkshire pigs were divided into 12 comparable groups of five pigs each with due consideration given to litter, weight, sex, breed and general thriftiness. In order to reduce within-group weight variation to a minimum, six of the groups were made up of pigs which had an average initial weight of approximately 48 lb. (Group A), while the remaining six groups were composed of pigs weighing approximately 37 lb. (Group B) initially. The groups from each of these two weight categories were then randomly allotted to the six treatments.

[1] The authors are grateful to the following concerns for supplying materials used in these studies: Buckeye Cotton Oil Co., Cincinnati, O., Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y., and Dawes Laboratories, Inc., Chicago, Ill. Acknowledgment is also due Mr. J. R. Danion for assistance in caring for the animals.

[2] Contribution from the Department of Animal Husbandry, Athens, Georgia. Journal Paper No. 26 of the College Experiment Station.

The pigs were housed in a barn with concrete-floored pens and had free access to outside concrete runs. Feed and water were provided by means of self-feeders and automatic waterers.

Weights of the pigs were recorded at two-week intervals, feed consumption being determined during each period. Duration of the experiment was 89 days.

Composition of the rations is shown in table 1. The oil meals used were all produced by the solvent process and analyzed approximately 45% crude protein (N x 6.25). The soybean oil meal was a blend from two

TABLE 1. COMPOSITION OF RATIONS FED IN EXPERIMENT I

Ration number	1	2	3	4	5	6
Ground yellow corn, lb.	72.5	72.5	72.5	72.5	72.5	72.5
Soybean oil meal, lb.	20.0	15.0	10.0	5.0	10.0	6.67
Peanut oil meal, lb.	...	5.0	10.0	15.0	...	6.67
"Degossypolized" cottonseed oil meal, lb.	...	...	...	...	10.0	6.66
Dehydrated alfalfa meal, lb.	5.0	5.0	5.0	5.0	5.0	5.0
Mineral mixture, lb.[a]	2.0	2.0	2.0	2.0	2.0	2.0
B-vitamin supplement, lb.[b]	0.1	0.1	0.1	0.1	0.1	0.1
Antibiotic B_{12} supplement, lb.[c]	0.5	0.5	0.5	0.5	0.5	0.5
Vitamins A and D_2[d]	+	+	+	+	+	+
	100.1	100.1	100.1	100.1	100.1	100.1
Calculated crude protein, %	15.9	16.0	16.0	16.1	16.0	16.0

[a] Mineral mixture supplied 1 lb. ground limestone, 0.5 lb. defluorinated phosphate, and 0.5 lb. salt mixture per 100 lb. of feed. Salt mixture contained 0.2 gm. cobalt sulfate and 20 gm. zinc sulfate per lb.

[b] Calculated to supply 2 mg. riboflavin, 4 mg. pantothenic acid, 9 mg. niacin, 10 mg. choline and 30 mcg. folic acid per lb. of feed, provided by Fortafeed 2–49c (Lederle).

[c] Calculated to supply 9 mg. chlortetracycline and 9 mcg. vitamin B_{12} per lb. of feed, provided by Aurofac (Lederle).

[d] Calculated to supply 500 I.U. vitamin A and 100 I.U. vitamin D per lb. of feed, provided by stabilized vitamin A and vitamin D_2 (Dawes).

sources. A "degossypolized" cottonseed oil meal was used, having 89.9% of its nitrogen soluble in 0.02N NaOH solution, and a free gossypol content of 0.02%.

The substitution ratios employed were: (1) soybean oil meal alone; (2) three parts soybean oil meal to one part peanut oil meal; (3) one part soybean oil meal to one part peanut oil meal; (4) one part soybean oil meal to three parts peanut oil meal; (5) one part soybean oil meal to one part "degossypolized" cottonseed oil meal. All rations were approximately equal in crude protein content.

Experiment II. Twenty-one Duroc weanling pigs were assigned to three comparable groups which were allotted at random to the treatments. All pigs were fed in concrete dry-lot with feed and water provided *ad libitum*. Weights and feed consumption were recorded at two-week intervals.

Composition of the rations fed is presented in table 2. The rations com-

TABLE 2. COMPOSITION OF RATIONS FED IN EXPERIMENT II

Ration number	1	2	3
Ground yellow corn	76.8	76.8	77.8
Soybean oil meal	19.0	...	...
"Degossypolized" cottonseed oil meal	...	19.0	15.0
Dehydrated alfalfa meal	2.0	2.0	2.0
Fish-soluble product [a]	...	...	3.0
Mineral mixture [b]	2.0	2.0	2.0
B-vitamin supplements [b]	0.1	0.1	0.1
Vitamin B_{12} supplement [c]	0.1	0.1	0.1
Vitamins A and D_2 [b]	+	+	+
	100.0	100.0	100.0

[a] A dry fish-solubles product including liver and glands, provided by F. S. Blend (Dawes).
[b] See footnote to table 1.
[c] Calculated to supply 10 mcg. vitamin B_{12} per lb. of feed, provided by Profactor-10 (Lederle).

pared were (1) a corn-soybean-oil-meal mixture (2) a corn-"degossypolized"—cottonseed oil-meal mixture and (3) the corn-"degossypolized"-cottonseed oil-meal mixture with a dried fish-solubles product replacing 3% of the cottonseed oil meal. The "degossypolized" cottonseed oil meal used in this experiment was from the same batch employed in Experiment I. Duration of the trial was 93 days.

Experiment III. This trial was conducted to compare soybean oil meal, peanut oil meal, "degossypolized" cottonseed oil meal and a blend of equal parts of these protein concentrates as sources of supplementary protein in rations fed to growing swine on pasture.

Forty weanling Duroc pigs were used. These were divided into four comparable groups which in turn were randomized to treatments. Each group of pigs was fed on a pasture plot one-half acre in size. Topography and herbage was similar on the various plots. The available forage was predominantly native grasses with some Ladino clover. Feed and water were provided *ad libitum*. Duration of the trial was 72 days.

TABLE 3. COMPOSITION OF RATIONS FED IN EXPERIMENT III

Ration number	1	2	3	4
Ground yellow corn	77.9	77.9	77.9	77.9
Soybean oil meal	20.0	...	...	6.67
"Degossypolized" cottonseed oil meal	...	20.0	...	6.67
Peanut oil meal	...	...	20.0	6.66
Mineral mixture [a]	2.0	2.0	2.0	2.0
Vitamin B_{12} supplement [b]	0.1	0.1	0.1	0.1
	100.0	100.0	100.0	100.0

[a] See footnote to table 1.
[b] See footnote to table 2.

Composition of the diets fed is shown in table 3. The crude protein content was calculated to be approximately 16.0% initially. This level was reduced to approximately 14.0% when the pigs averaged 125 lb. in each group.

Results and Discussion

A summary of the average performance data of the pigs from Experiment I is presented in table 4. The data were analyzed by analysis of variance.

When the combined data of both groups for the entire experiment was considered, no significant difference was found between treatments with respect to average daily gain or efficiency of feed utilization. There was also no significant difference between weight groups or treatment X weight group with respect to daily gain.

However, when the data for the two weight groups are considered separately it reveals a somewhat faster average growth rate for those pigs receiving rations 1 and 3 in group A; a trend that was observed to be consistent throughout the experiment. This trend was not apparent with the pigs receiving these rations in group B. The slightly slower gain that is apparent for group A on ration 5 is due to the poor performance of one pig in that group. If the data of this pig were eliminated then the performance for this group was similar to that of the other groups. Since the pig was thrifty in appearance and showed no gross pathology its data were not removed.

It was observed that pigs in both weight groups receiving ration 4 gained at a slower rate during the first 56 days of the trial. However, by the end of the experiment the performance of these groups had improved considerably. The reasons for this change are not readily apparent although a change in the amino acid make-up or the increased caloric density of the ration as a result of the shift from a 16% protein ration to a 14% protein ration at about this time, or both, may have exerted some influence in this regard.

Calculation of the amino acid content of the various rations using values reported in the literature (Williams, 1950; Lyman *et al.*, 1949), indicate that the methionine-cystine content of all rations used in Experiment I would be marginal relative to the requirements suggested by Curtin *et al.* (1952) and Becker *et al.* (1955). Lysine content was also marginal as calculated for rations 2, 3, 4, 5 and 6 relative to the requirement indicated by Mertz *et al.* (1949). The methionine and lysine levels were slightly lower on the 14% protein rations used during the latter part of the experiment.

Analysis of the growth data from Experiment II revealed no significant difference between treatments. Slightly faster gains were obtained on the corn-"degossypolized"-cottonseed oil-meal ration supplemented with fish solubles (ration 3) than on the corn-soybean-oil-meal ration (ration 1)

TABLE 4. AVERAGE PERFORMANCE DATA FOR PIGS ON EXPERIMENT I

Ration number	1		2		3		4		5		6	
Weight group	A	B	A	B	A	B	A	B	A	B	A	B
No. of pigs	5	5	5	5	5	5	5	5[a]	5	5	5	5
Av. initial wt., lb.	48.2	36.6	48.0	37.0	48.4	37.0	47.6	36.6	46.8	36.8	47.2	37.0
Av. final wt., lb.	206.2	177.6	187.2	175.8	209.0	180.4	195.8	177.9	173.4	184.4	200.2	172.0
Av. daily gain, lb.	1.78	1.58	1.56	1.56	1.80	1.61	1.67	1.59	1.42	1.66	1.72	1.52
Av. feed per lb. gain, lb.	3.24	3.22	3.59	3.51	3.83	3.22	3.45	3.58	3.73	3.16	3.58	3.54
Av. daily feed intake, lb.	5.76	5.09	5.61	5.48	6.89	5.18	5.76	5.73	5.30	5.25	6.16	5.38

[a] One pig removed due to a spinal injury. A missing plot value was calculated for this pig.

TABLE 5. AVERAGE PERFORMANCE DATA OF PIGS ON EXPERIMENT II

Ration number	1	2	3
Number of pigs	7	7	7
Av. initial wt., lb.	52.6	52.3	52.4
Av. final wt., lb.	190.1	187.6	196.3
Av. daily gain, lb.	1.48	1.45	1.55
Feed required per lb. gain, lb.	3.53	3.79	3.41

or the corn-"degossypolized"-cottonseed oil-meal ration (ration 2). Greater efficiency of feed conversion was also obtained on ration 3 (table 5).

Dyer *et al.* (1952) reported a severe incidence of dermatitis with pigs fed rations similar to rations 2 and 3. Raper and Curtin (1953) reported that additions of zinc and cobalt prevented this condition on similar diets. More recently Tucker and Salmon (1955) and Luecke *et al.* (1956) have indicated that this condition can be prevented by added dietary zinc. No difficulty of this nature was experienced in this trial in which approximately 23 P.P.M. of zinc were added to the ration. Growth response was essentially the same for pigs receiving the corn-soybean-oil-meal ration (ration 1) and the corn-"degossypolized"-cottonseed oil-meal ration (ration 2, table 6).

In Experiment III, gains for pigs receiving the corn-peanut-oil meal ration (ration 3) were consistently slower throughout the trial than those of pigs receiving the other three rations. However, this difference was not significant when tested statistically. Likewise, there was no significant difference between the other treatments on this trial. It may be noted that the "degossypolized"-cottonseed oil-meal ration (ration 2) supported the most rapid gains of any of the treatments employed in Experiment III.

Summary and Conclusions

Three experiments were conducted to study the value of soybean oil meal, peanut oil meal and "degossypolized" cottonseed oil meal when used singly and in various combinations to supply the supplementary protein in growing swine rations.

The combined data for Experiment I revealed no significant difference

TABLE 6. AVERAGE PERFORMANCE DATA OF PIGS ON EXPERIMENT III

Ration number	1	2	3	4
Number of pigs	10	10	10	10
Av. initial wt., lb.	51.7	50.8	51.6	51.6
Av. final wt., lb.	164.8	172.2	158.4	166.8
Av. daily gain, lb.	1.57	1.69	1.48	1.60
Feed required per lb. gain, lb.	3.82	3.51	3.41	3.97

in rate of gain for pigs receiving rations in which peanut oil meal was used to replace varying amounts of soybean oil meal up to three-fourths of the total supplementary protein. Pigs receiving a ration in which one-half of the soybean oil meal was replaced by "degossypolized" cottonseed oil meal and one containing a blend of equal parts of soybean oil meal, peanut oil meal and "degossypolized" cottonseed oil meal as sources of protein performed equally as well as did pigs receiving soybean oil meal alone.

In Experiment III there was no significant difference for pigs self-fed rations on pasture which contained soybean oil meal, degossypolized" cottonseed oil meal, peanut oil meal or a blend of equal parts of these three ingredients as the source of supplementary protein.

Under the conditions imposed in these studies it appears that at least one-half of the soybean oil meal can be satisfactorily replaced by peanut oil meal in dry-lot rations for growing pigs when non-protein dietary factors are in adequate supply. "Degossypolized" cottonseed oil meal appeared to be a satisfactory source of supplementary protein for growing swine when fed in dry-lot and on pasture.

Literature Cited

Becker, D. E., A. H. Jensen, S. W. Terrill and H. W. Norton. 1955. The methionine-cystine need of the young pig. J. Animal Sci. 14:1086.

Blaylock, L. G., and L. R. Richardson. 1950. Peanut oil meal, mixtures of soybean and cottonseed and mixtures of soybean and peanut meals as sources of protein for baby chicks. Poultry Sci. 29:656.

Curtin, L. V., J. K. Loosli, J. Abraham, H. H. Williams and L. A. Maynard. 1952. The methionine requirement for the growth of swine. J. Nutr. 48:499.

Dyer, I. A., J. T. Harrison, W. S. Nicholson, Jr. and A. E. Cullison. 1952. Penicillin, lysine, methionine and fish solubles supplement a corn-degossypolized cottonseed meal ration for weanling pigs. J. Animal Sci. 11:465.

Luecke, R. W., J. A. Hoeffer, W. S. Brammell and F. Thorp, Jr. 1956. Mineral interrelationships in parakeratosis of swine. J. Animal Sci. 15:347.

Lyman, C. M., and K. A. Kuiken. 1949. The amino acid composition of meat and some other foods. Texas Agr. Exp. Sta. Bul. 708:21.

Mertz, E. T., D. C. Shelton and W. M. Beeson. 1949. The amino acid requirements of swine, lysine. J. Animal Sci. 8:524.

Raper, J. R., and L. V. Curtin. 1953. Proceedings of third conference on processing as related to nutritive value of cottonseed meal, p. 17.

Tucker, H. F. and W. D. Salmon. 1955. Parakeratosis or zinc deficiency disease in the pig. Proc. Soc. Exptl. Biol. Med. 88:613.

Wallace, H. D., T. J. Cunha and G. E. Combs. 1955. Low-gossypol cottonseed meal as a source of protein for swine. Florida Agr. Exp. Sta. Bul. 566:20.

Williams, H. H. 1950. Studies of the amino acid composition of feedstuffs. Proc. Cornell Nutr. Conf. for Feed Mfg., 76.

EFFECT OF PROTEIN LEVEL IN THE RATION ON GOSSYPOL TOLERANCE IN GROWING-FATTENING PIGS

FRED HALE AND CARL M. LYMAN [1]

Departments of Animal Husbandry and Biochemistry and Nutrition
Texas Agricultural Experiment Station [2]

DURING the last few years specially processed cottonseed oil meals of low free gossypol content have been made available commercially through two general manufacturing processes. In the first of these, an aromatic amine is used to inactivate the gossypol. The second process is the prepress-solvent method, by which the free gossypol content of the meal can be reduced to low levels without excessive heat which might reduce the nutritional value of the protein. Such meals are sometimes called degossypolized cottonseed oil meals. They have been successfully used by a number of investigators as the sole protein supplement in swine rations (Dyer *et al.*, 1951) (Wallace *et al.*, 1953) (Robinson, 1955).

That cottonseed meal with a high free gossypol content is toxic to swine has been known for a number of years (Hale, 1930; Robinson, 1934). It has been shown that the effects of gossypol can be prevented by adding iron salts (Withers and Brewster, 1913; Fletcher, 1948; Barrick, 1950) to the ration, but this solution to the problem has not been generally accepted by swine producers.

The work of Hale (1930) showing that commercial hydraulic meal is safe if the amount is limited to 9% of the ration has proved to be a very useful guide for effective use of cottonseed oil meal in swine rations. Since 9% of cottonseed oil meal does not supply enough protein to balance the ration, a second protein supplement is required.

The production and use of cottonseed oil meal which is always safe at any levels fed in the ration without the addition of iron salts or other additives for gossypol detoxification, is a more ideal solution. If degossypolized cottonseed oil meals are to be extensively used in swine rations, it is essential to have reliable information concerning the free gossypol tolerance level in swine. Many of the older reports on feeding cottonseed oil meal to swine do not include adequate descriptions with respect to the gossypol content of the meals used. No experiments showing the effects of graded levels of free gossypol on young pigs could be found in the literature.

The purpose of this communication is to report the results of experiments designed to give this information. One of the most characteristic chemical properties of gossypol is its reactivity with amino groups. Thus

[1] Post mortem examinations of pigs which died during the experiments were conducted by Harold E. Redmond. The authors wish to express their appreciation to Dr. Philip D. Aines and the Buckeye Cotton Oil Division of the Buckeye Cellulose Corporation for the pilot plant preparation of solvent extracted cottonseed oil meal of high free-gossypol content.

[2] Texas A. & M. College System, College Station.

when gossypol is inactivated by heat treatment during processing it is postulated that this occurs by reaction of the gossypol with an amino group of the protein and the formation of an insoluble indestible complex (Clark, 1928) (Baliga and Lyman, 1956). The accumulation of fluid in the body cavity as a result of feeding gossypol suggests that part of the mechanism of gossypol toxicity involves interference in protein metabolism (Ferguson, 1956). Such interference might vary with the quantity and quality of the protein in the ration. For this reason, experiments with more than one level of protein in the diet are included in the present report.

Experimental

Animals. The pigs used in these tests were purebred Durocs bred and raised by the Texas Agricultural Experiment Station. They were from 80 to 100 days old when placed on test, and represented the kind and weight of pigs often purchased as feeder pigs for commercial feeding. The pigs

TABLE 1. COMPOSITION OF RATIONS FOR EXPERIMENT II

	15% Protein rations [a]				30% Protein rations [a]			
Ration No.	1	2	3	4	5	6	7	8
Cottonseed oil meal, lb.	0	1.6	3.2	4.8	0	1.6	3.2	4.8
Soybean oil meal, lb.	13.0	11.8	10.8	8.0	52.4	51.4	50.0	47.4
Milo, lb.	81.0	80.6	80.0	81.2	41.6	41.0	40.8	41.8
Alfalfa leaf meal, lb.	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Limestone, lb.	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Salt, lb.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Aurofac, lb.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

[a] Rations were analyzed for crude protein content.

had been treated for cholera and for round worms (ascarids) before placing on test. Both barrows and gilts were used in the test. The pigs were kept and fed in individual pens with concrete floors and with an outside concrete pen adjacent to the pen inside the feeding shed. Both the inside and outside pens were 4 feet by 8 feet in size. Each pen inside the barn contained an individual self-feeder and each outside pen contained an individual water trough. The pens were cleaned and washed daily.

Rations for Experiment I. The rations for all groups except the control group No. 1, contained 76.5% milo, 9% mixed cottonseed oil meal, 9% soybean oil meal, 3% alfalfa leaf meal, 1.5% limestone, 0.5% salt and 0.5% Aurofac containing chlortetracycline and vitamin B_{12}.

The indicated free gossypol level was obtained by mixing two meals; one containing 0.014% free gossypol and the other 0.317%.

The control ration contained 18% soybean oil meal and not cottonseed oil meal.

Rations for Experiment II. The rations used for Experiment II are given in table 1. Free gossypol was supplied in the form of hexane extracted cottonseed meats prepared in a commercial laboratory with a minimum of

heat. The meal contained 0.64% free gossypol and 38.25% protein. The protein content of the soybean oil meal was 47.94%.

Weekly weights and feed consumption of the pigs were recorded. Pigs that died were posted by Harold E. Redmond of the School of Veterinary Medicine.

Results and Discussion

Table 2 shows the results of feeding rations containing free gossypol levels ranging from 0 to 0.028% of the ration. These levels were obtained by mixing two meals of widely divergent free gossypol content. In order to minimize any possible effects of variation in protein quality, soybean oil meal was used to supply one-half of the protein supplement in each group except in group 1 where only soybean oil meal was used.

All animals which received 0.01% free gossypol or less showed no gossypol toxicity symptoms and the gain was as good or almost as good as in the control group (Group 1) which received the all soybean protein supplement. With 0.015% free gossypol in the diet, there were no death losses, but two pigs showed gossypol toxicity symptoms as indicated by severe thumping or labored breathing. This labored breathing was due to partial congestion of the lungs with fluid.

Above .015% free gossypol death occurred in all groups. Post-mortem examination of the pigs which died showed the following macroscopic lesions: excessive quantities of a serous fluid in the pleural and peritoneal cavities; heart, dilated and flabby; lungs, congested and edematous; liver, enlarged and passively congested; spleen, congested; kidneys, congested; lymph glands, when affected, congested and swollen.

It is of interest that in this experiment half and half cottonseed oil meal and soybean oil meal produced just as good gains as did soybean oil meal alone when low gossypol cottonseed oil meal was used, (Group 2).

Experiment II was designed to determine whether the level of protein in the diet would alter the gossypol tolerance level, (table 3). With a 15% crude protein diet, .01% free gossypol did not produce any gossypol toxicity symptoms, but it gave slightly lower growth rates as compared with the control group receiving no gossypol. Levels of free gossypol above 0.01% resulted in toxicity symptoms and the death of some animals in each group.

In sharp contrast there was not a single animal receiving the 30% protein ration which showed any symptoms of gossypol toxicity whatsoever. When these animals were slaughtered at the termination of the experiment, they were examined by a qualified veterinarian and no internal symptoms could be found.

Table 4 shows the amount of gossypol consumed by individual animals during the test period. Animals which died on the 15% protein ration had consumed up to 39.6 gm. gossypol. Every animal in the groups fed 30%

TABLE 2. EFFECT OF FREE GOSSYPOL LEVEL IN THE DIET ON GROWING-FATTENING PIGS

Experiment I, (70-day test) Dec. 29, 1954–Mar. 9, 1955

	Crude protein level of diets, 15.5%								
	Percent gossypol in diet								
	None	.0013	.0025	.007	.010	.015	.019	.022	.028
Group No.	1	2	3	4	5	6	7	8	9
No. of animals [a]	8	8	8	8	8	8	8	8	8
Initial wt., lb.	57.6	57.6	57.8	57.6	57.6	57.7	57.6	57.7	57.7
Final wt., lb.	192.4	192.4	181.3	187.3	188.8	184.8	178.7[b]	185.8[b]	161.6[b]
Ave. daily gain, lb.	1.92	1.92	1.76	1.85	1.87	1.81	1.70[b]	1.83[b]	1.52[b]
Feed per 100 lb. gain, lb.	367.4	377.5	374.0	348.2	364.2	361.7	364.5	351.4	373.8
No. of deaths	None	None	None	None	None	None	1	2	2
No. of animals which lived, but showed gossypol toxicity symptoms	None	None	None	None	None	2	2	None	4

[a] Pigs fed in individual pens.
[b] For animals remaining at close of test.

TABLE 3. EFFECT OF PROTEIN LEVEL IN THE RATION ON GOSSYPOL TOLERANCE IN GROWING-FATTENING PIGS

Experiment II, (84 day test) Dec. 28, 1955–Mar. 21, 1956

	Standard protein level, 15% C.P. % Gossypol in diet				High protein level, 30% C.P. % Gossypol in diet			
	0	.01	.02	.03	0	.01	.02	.03
Ration No.	1	2	3	4	5	6	7	8
No. of animals [a]	8	8	8	8	8	8	8	8
Initial wt., lb.	58	58	58	58	58	58	58	58
Final wt., lb.	204	190	170[b]	135[c]	188	191	186	177
Av. daily gain, lb.	1.74	1.57	1.31	1.16	1.55	1.58	1.52	1.42
Feed per 100 lb. gain, lb.	366	376	424	461	387	379	381	384
No. of deaths	None	None	2	4	None	None	None	None
No. of animals which lived, but showed gossypol toxicity symptoms	None	None	3	2	None	None	None	None

[a] Pigs fed in individual pens.
[b] 6 remaining animals.
[c] 4 remaining animals.

protein rations and either .02% or .03% gossypol in the ration consumed more gossypol than did any of those which died on the 15% protein ration.

Summary

Restriction of the free gossypol content of the ration to 0.01% or less provided a safe level for feeding cottonseed oil meal to growing-fattening pigs. Such rations may be self-fed in self-feeders with good results.

Although levels of free gossypol above 0.01% resulted in toxicity symptoms and death of some of the pigs in each group, none of the pigs

TABLE 4. GOSSYPOL CONSUMED BY INDIVIDUAL PIGS

Experiment II

15% Protein ration				30% Protein ration			
.02% Gossypol		.03% Gossypol		.02% Gossypol		.03% Gossypol	
Pig No.	Total gossypol consumed	Pig No.	Total gossypol consumed	Pig No.	Total gossypol consumed	Pig No.	Total gossypol consumed
	gm.		gm.		gm.		gm.
93	51.8	23[b]	29.6	30	51.1	80	65.4
3[a]	35.7	51[c]		50	45.4	40	62.9
104[b]	39.6	20[a]	26.4	21	45.2	79	64.4
39[a]	31.1	130[b]	35.6	81	40.9	70	57.1
106	53.6	75[a]	44.5	125	44.9	74	62.0
151	51.6	55	54.5	46	43.2	87	71.4
44[a]	28.9	68	52.3	123	42.7	133	66.2
110[b]	24.9	36[b]	24.0	126	41.8	77	50.9
Av.	39.7		38.1		44.4		62.5

[a] Animals which showed gossypol toxicity symptoms but did not die.
[b] Animals which died with symptoms of gossypol poisoning.
[c] Removed from lot 3 weeks after test started because of leg injury and general unthriftiness.

receiving the 30% protein ration showed any symptoms of toxicity whatsoever.

Animals which died on the 15% protein ration consumed up to 39.6 gm. of free gossypol. Every pig in the groups fed 30% crude protein ration (and with either 0.02% or 0.03% free gossypol in the ration) consumed more free gossypol than did any of the pigs which died on the 15% crude protein ration.

When the low free gossypol cottonseed oil meal was fed, the pigs gained as well on the half cottonseed oil meal-half soybean oil meal supplement as did the pigs on the soybean oil meal alone supplement.

Literature Cited

Baliga, B. P. and Carl M. Lyman. Preliminary report on the nutritional significance of bound gossypol in cottonseed meal. J. Amer. Oil Chem. Soc. (in press).

Barrick, E. R., F. H. Smith, G. Matrone, H. A. Steward and G. H. Wise. 1950. Some factors affecting the nutritive value of cottonseed meal for swine. J. Animal Sci. 9:646.

Clark, E. P. 1928. The nature of Carruth's D gossypol. J. Biol. Chem. 76:229.

Dyer, I. A., A. E. Cullison, and W. J. Hays. 1951. Special solvent cottonseed meal with and without a vitamin B_{12} supplement and streptomycin as a source of protein for pigs. J. Animal Sci. 10:624.

Ferguson, T. M. 1956. Unpublished observations.

Fletcher, J. L. 1948. Controlling cottonseed meal poisoning in pigs. Miss. Farm Research, 11:4.

Gray, Dan T. 1917. Feeding hogs in the South. U. S. Dept. Agr. Farmers Bul. 411.

Hale, Fred. 1930. Cottonseed meal as a feed for hogs. Texas Agr. Exp. Sta. Bul. 410.

Marshall, F. R. 1905. Feeding fermented cottonseed meal to hogs. Tex. Agr. Exp. Sta. Bul. 78.

Robison, W. L. 1934. Cottonseed meal for pigs. Ohio Agr. Exp. Sta. Bul. 534.

Robison, W. L. 1955. Swine experiments. Ohio Agr. Exp. Sta. Animal Science Mimeograph Series No. 95.

Wallace, H. D., L. T. Albert, W. A. Ney, G. E. Combs and T. J. Cunha. 1953. Effects of reducing and discontinuing aureomycin supplementation during the growing-fattening period of pigs fed corn-peanut meal, corn-soybean, and corn-cottonseed meal rations. J. Animal Sci. 12:316.

Withers, W. A. and J. F. Brewster. 1913. Studies on cottonseed meal toxicity. II. Iron as an antidote. J. Biol. Chem. 15:161.

THE EFFECT OF AMYLASES ON THE DIGESTIBILITY OF STARCH BY BABY PIGS [1]

H. M. CUNNINGHAM AND G. J. BRISSON [2]

Canada Department of Agriculture

ECONOMICALLY, it would be advantageous if starch could substitute for lactose in sow's milk replacements for newborn pigs. This carbohydrate does not permit satisfactory growth of two-day old pigs (Cunningham and Brisson, 1955) but appears to be well utilized by pigs removed from the sow at seven days of age (Becker *et al.*, 1954). Glucose and lactose are readily utilized by baby pigs and have been widely used as carbohydrates in experimental diets. Various workers (Johnson, 1949; Becker *et al.*, 1954), have observed that the newborn pig responds to sucrose and fructose with severe diarrhea, rapid weight loss, unthriftiness and death. Becker *et al.* (1954) observed that by seven days of age, 60% of the baby pigs were able to utilize sucrose. They found no difference in the nutritive value of dextrose, lactose, dextrin, or corn starch for pigs 7 to 35 days of age.

Kitts *et al.* (1956) reported that pancreatic amylase activity of pigs increased from negligible levels at birth to high levels after 21 days. Further work by Bailey *et al.* (1956) indicated that intestinal maltase and sucrase activity were also low at birth and increased with age. Intestinal lactase activity, on the other hand, was of a high order at birth and declined after two weeks of age. If baby pigs are deficient in amylases, the work of Ivy *et al.* (1936) suggested that these enzymes may be administered orally. It was demonstarted that a large proportion of amylases ingested experimentally by humans escaped gastric digestion and was recovered in the intestine.

The present work was undertaken to study the effect of supplemental amylolytic enzymes on the digestibility and utilization of starch by baby pigs. The effect of cooking the starch and the susceptibility of cooked starch to hydrolysis by supplemental amylases were also studied.

Experimental

Trial 1. Eight litter-mate Yorkshire x Landrace-Chester two-day-old baby pigs were allotted on an equal weight basis into two groups of four. One group received Diet 1 (table 1) and the other group Diet 1 plus the diet. The procedure followed in preparing the purified diet was similar

[1] Contribution No. 337, Chemistry Division, Science Service, Ottawa, Ontario.

[2] The authors wish to thank F. K. Kristjansson of the Animal Husbandry Division, Central Experimental Farm, for supplying the baby pigs, and R. G. Sample for assisting in their care. The chlortetracycline was supplied by the courtesy of T. H. Jukes, American Cyanamid Co., Pearl River, New York, U.S.A.

enzymes. Pancreatic amylase (1–50–5 min.), malt amylase and pancreatin [3] were each added at a level of 1.0% of the starch content of to that reported in previous work (Cunningham and Brisson, 1955). The starch was incorporated as a suspension and the liquid diet was stirred thoroughly before feeding. In order to reduce enzymatic hydrolysis of starch before ingestion by the pig, enzymes were not added until the time of feeding.

Pigs were fed six times daily from metal troughs, the efficiency of which was reported earlier. The diet was weighed to the nearest gram and each pig was fed to appetite consistent with the absence of severe digestive disturbances. Feces were collected four times daily beginning on the second day of the experiment. Earlier described methods were followed in pre-

TABLE 1. COMPOSITION OF DIET 1

Ingredient	Gm. per kg. of fluid diet
Corn starch	50.0
Crude casein	40.0
Lard [a]	28.0
Lecithin	2.0
Minerals [b]	9.9
Vitamins [b]	
Chlortetracycline	10 mg.

[a] The lard was homogenized with the lecithin before adding it to the diet.
[b] Same as those used previously (Cunningham and Brisson, 1955).

serving the feces and in the analysis of dry matter, protein, fat and ash. Fecal carbohydrates was calculated by difference. Duration of the experiment was four weeks with two successive 7-day collection periods during the first two weeks. All pigs were weighed weekly.

Trial 2. This experiment was initiated to determine if cooking improved the digestibility of starch and if supplemental amylases assisted in its digestion.

It is well known that most amylase preparations have extremely weak action on raw starch (Sumner and Myrback, 1951). ***In vitro*** experiments indicated that the amylolytic enzymes employed in this study would not hydrolyze raw starch unless it was first boiled. Suspensions of raw starch, injected into ligated loops of rats' intestines kept ***in situ,*** were quickly hydrolyzed but water extracts of the intestinal mucosa would hydrolyze starch rapidly only if the starch was first boiled. All the factors responsible for the utilization of raw starch are not known, but in view of the above observations, it seems possible that the baby pig may not be able to alter starch in such a way that it can be attacked by the amylolytic enzymes. It was thought that perhaps cooking the raw starch would prepare it for

[3] The enzymes were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

the hydrolytic action of the amylases and improve its utilization by baby pigs.

Twelve pigs from a large litter were allotted according to weight into three equal groups. Each group was assigned at random to one of the following treatments; raw starch (Group I), cooked starch (Group II), and cooked starch plus amylases (Group III). The cooked starch was prepared by heating a 10% starch suspension in a boiling water bath for 30 minutes with frequent stirring. The mixture was cooled to approximately 50° C. and the other ingredients added. The enzyme supplemented diet was prepared by adding just prior to feeding, 1 gm. of pancreatic amylase and 1 gm. of malt amylase per 100 gm. of starch. The pigs were fed as much as they could consume without displaying severe diarrhea. The incidence of diarrhea noted for each pig was recorded. Feces collections began on the second day and lasted for four successive periods of 3, 4, 7 and 14 days.

Results

Trial 1. The data in table 2 indicate that the digestibility of the starch in both diets was over 99%. There was also no difference in the total quantity of feed consumed by the two groups of pigs. The amylolytic enzymes, therefore, had no effect on the coefficient of digestibility of the starch or on the total quantity of starch digested. The digestibility of protein, fat and ash was also unaffected by enzyme supplementation.

The feed consumption was low; all pigs appeared unthrifty and grew slowly. One animal on Diet I died during the third period but its death was attributed to congestion of the lungs. The condition of the pigs began to improve after two weeks and by the end of the trial they were in satisfactory condition.

Trial 2. Data on feed consumption, weight gains, incidence of diarrhea and digestibility are given in table 3. The response of the pigs to the raw-starch diet was similar to that in Trial 1. A general appearance of rough coat and unthriftiness became apparent by the third day. All pigs lost weight during the first week and weight gains thereafter were not large. Some restriction of feed was required to avoid diarrhea but the digestibility of the starch was still close to 100%.

The growth and general appearance of the pigs in group 2 was not improved by cooking the starch. One pig died on the ninth day and the remaining pigs appeared to be even less thrifty and more difficult to raise than those receiving raw starch. There was no difference in the digestibility of the cooked and raw starch in periods 1 and 4 but the digestibility of the cooked starch was significantly lower ($P<0.01$) during periods 2 and 3. The weight gains of the three surviving pigs in Group 2 were slightly but not significantly lower than those of pigs in Group 1. This is in agreement with the work of Catron (1956) in which a faster growth rate was obtained with raw than with cooked starch.

TABLE 2. AVERAGE WEIGHT GAINS AND FEED UTILIZATION OF PIGS IN TRIAL 1

No. of pigs	Period No.[a]	Dry matter intake per day	Av. daily gain	Av. days of scours per pig	Apparent digestibility				
					Dry matter	Protein	Fat	Starch	Ash
		gm.	gm.		%	%	%	%	%
				Group 1 Controls (Av. initial wt., 1276 gm.)					
4	1	45	28	0.5	96.9	94.4	95.2	98.9	94.4
4	2	60	50	1.2	95.7	93.6	91.2	100.3	87.9
3	3	101	85	0.0					
				Group 2 Amylases (Av. initial wt., 1280 gm.)					
4	1	43	20	1.7	96.7	94.6	93.4	99.9	91.4
4	2	69	58	1.0	95.2	94.4	87.3	99.7	87.7
4	3	98	67	0.3					

[a] Periods 1 and 2 were 7 days each and period 3 was 14 days.

TABLE 3. AVERAGE WEIGHT GAINS AND FEED UTILIZATION OF PIGS IN TRIAL 2

No. of pigs	Period No.[a]	Av. D.M. intake per day	Av. daily gain	Av. days of scours per pig	Apparent digestibility				
					Dry matter	Protein	Fat	Starch	Ash
		gm.	gm.		%	%	%	%	%
				Group 1 raw starch (Av. initial wt., 1460 gm.)					
4	1	39		0.00	98.6	98.3	98.3	98.8	98.0
4	2	36	−27	0.75	92.6	86.4	88.7	98.3	86.3
4	3	69	69	0.25	92.8	91.1	85.7	99.1	83.3
4	4	156	146	1.00	97.5	97.4	98.1	97.7	93.0
				Group 2 cooked starch (Av. initial wt., 1450 gm.)					
4	1	33		0.00	98.5	98.2	98.2	99.0	97.8
4	2	40	−33	0.50	91.5	88.8	92.0	92.7	85.3
3	3	68	53	0.33	92.8	91.8	94.8	93.1	83.5
3	4	150	129	1.00	97.6	97.4	97.5	98.3	94.2
				Group 3 cooked starch + Enzymes (Av. initial wt., 1450 gm.)					
4	1	31		0.00	97.0	96.3	97.0	98.0	96.5
4	2	34	−36	0.75	91.5	88.1	89.3	95.1	83.1
2	3	45	26[b]	1.00	91.5	86.4	88.0	98.5	79.0
1	4	74	—	3.00	87.4	79.5	80.5	97.8	75.8

[a] Period 1–3 days, Period 2–4 days, Period 3–7 days, Period 4–14 days.
[b] Weight gain on only one pig.

None of the pigs receiving the cooked starch diet plus enzymes survived past the fourth week. Their performance in regard to both feed intake and weight gains was below that of the pigs on the other two diets. Feed refusals occurred more frequently, but the digestibility of the feed during the first three periods was as high as that recorded by the pigs on diets 1 and 2. Just prior to death, the last surviving pig in group 3 displayed considerable diarrhea. As a result, the digestion coefficients of the group for this period were quite low. After the first week, when the enzymes did not appear to be affecting the utilization of starch, the level of amylases in the diet of the two remaining pigs was doubled. It is not known if this could have contributed to the further feed refusals and deaths of these pigs.

Digestibility of all fractions of the diet was higher during the first three days than during the following four days. This may be explained by the fact that feed refusals and scouring did not occur until the fourth day. The volume of feces during diarrhea was seldom large, but whenever the feces were fluid in consistency, feed was withheld for several hours. The data on dry matter digestibility indicate that the quantity of undigested material was seldom high and that scouring was probably not the predominant factor contributing to the death of the pigs.

Discussion

It was somewhat surprising to find that while the pigs did not thrive on the starch diets, the starch was almost 100% digested. The coefficients of digestibility of the starch could not have been increased further by enzyme supplementation but the total quantity of starch digested per unit of time was subject to considerable improvement. No such benefits were observed from supplemental enzyme activity. The orally administered amylases are either destroyed before they reached the portion of the gastrointestinal tract where pH and other conditions are favorable to their action, or their substrate, starch may require additional treatment with other enzymes or materials for its complete breakdown to glucose.

Bailey *et al.* (1956) reported that at birth, the intestine of the baby pig secretes only negligible amounts of maltase. One might expect that once the starch was hydrolyzed by amylase to maltose, the breakdown could proceed no further without supplemental maltase. Additional experiments performed in our laboratory (to be published later) show that the baby pig can utilize some maltose at birth and may be raised almost as well on maltose as on glucose from one day of age.

Larner and McNickle (1955) reported that oligo-1,6-glucosidase isolated from hog intestinal mucosa was required along with alpha-amylase and maltase for the essentially complete digestion of starch in the gastrointestinal tract. However, preliminary experiments here indicate that supplementing the starch diets of one-day old baby pigs with fresh extracts of hogs' intestinal mucosa results in no improvement of the diet.

Malt amylase acts over a broad pH range of from 2 to 8 with an optimum pH of around 5 (Sumner and Myrbäck, 1951). It was expected that the acid pH of the baby pigs' stomach would be conducive to the action of this enzyme and that considerable starch would be broken down before the food left the stomach. The reason for the failure of the malt amylase to improve the utilization of starch is the subject of further investigation.

Summary

Experiments were performed with two-day-old baby pigs to observe the effect of supplementing various purified starch-containing diets with amylolytic enzymes. Supplemental pancreatic and malt amylases had no effect on growth rate, survival time, or digestibility of raw or cooked starch. Cooked starch appeared to be inferior to raw starch as a source of carbohydrate in baby pig diets.

Literature Cited

Bailey, C. B., W. D. Kitts and A. J. Wood. 1956. The development of the digestive enzyme system of the pig during its pre-weaning phase of growth; B. Intestinal lactase, sucrase and maltase. Can. J. Agr. Sci. 36:51.

Becker, D. E., E. E. Ullrey and S. W. Terrill. 1954. A comparison on carbohydrates in a synthetic milk diet for the baby pig. Arch. Biochem. 48:178.

Catron, Damon V. 1956. New developments in baby pig nutrition. Feedstuffs, May 5, pp. 50.

Cunningham, H. M. and G. J. Brisson. 1955. The utilization of lard by baby pigs. Can. J. Agr. Sci. 35:371. Also unpublished data.

Ivy, A. C., C. R. Schmidt and J. M. Beazell. 1936. On the effectiveness of malt amylase on the gastric digestion of starches. J. Nutr. 12:59.

Johnson, S. R. 1949. Comparison of sugars in the purified diet of baby pigs. Proc. Fed. Am. Soc. Expt. Biol. 8:387.

Kitts, W. D., C. B. Bailey and A. J. Wood. 1956. The development of the digestive enzyme system of the pig during its pre-weaning phase of growth; A. Pancreatic amylase and lipase. Can. J. Agr. Sci. 36:45.

Larner, J. and C. M. McNickle. 1955. Gastrointestinal digestion of starch. I. The action of oligo–1,6–glucosidase on branched saccharides. J. Biol. Chem. 215:723.

Sumner, J. B. and K. Myrbäck. 1951. The Enzymes, 1: part 1. Academic Press Inc., New York.

THE RELATION BETWEEN CERTAIN CHARACTERISTICS OF FATS AND OILS AND THEIR APPARENT DIGESTIBILITY BY YOUNG PIGS, YOUNG GUINEA PIGS AND PUPS

L. E. Lloyd and E. W. Crampton

Department of Nutrition, Macdonald College (McGill University) P.Q. Canada [1]

RELATIVELY few characteristics of fats and oils have been shown to have a definite influence on their utilization by the animal body. Langworthy and Holmes (1915) and Holmes and Deuel (1921) indicated that an inverse relationship exists between melting point and digestibility for fats melting at temperatures above 50° C. The more recent work of Hoagland and Snider (1943a) does not support the thesis that a definite relationship exists between the melting point of a fat and its digestibility. Those authors suggest that some other factor, possibly the stearic acid content, determines fat digestibility. However the experiments of Mattil and Higgins (1945) do not support this latter observation.

As a result of the rat experiments of Hoagland and Snider (1943b), and Cheng *et al.* (1949), it appears that, in general, the saturated fatty acids are less efficiently utilized than the unsaturated fatty acids.

The recent findings with rapeseed oil influenced the approach taken to the research to be described herein. That rapeseed oil, with its content of 40 to 50% trierucin, is poorly digested by the rat is well known, (Deuel *et al.*, 1948; Thomasson, 1955a, 1955b, 1956; Thomasson and Boldingh, 1955). Since the low digestibility of rapeseed oil has been attributed to a failure of absorption of the long chain fatty acid, erucic acid, it seemed pertinent to examine the effect of fatty acid chain length (or mean molecular weight) on the apparent digestibility of fats or oils.

Experimental

One hundred and four pigs, 87 guinea pigs and 75 pups, weaned at 14, 3, and 10 days respectively, were used in these feeding trials. Since at this age these animals normally obtain most of their energy from milk fat, this appeared to be an ideal stage of development to study fat utilization.

Twenty different fats or oils were added individually to a low-fat basal ration appropriate to the species involved. All fat or oil additions were made at the 20% level, and the apparent digestibility of each fat or oil was determined for each animal in the case of the guinea pigs and pups, and for each group of four animals in the case of the pigs.

The chromic oxide indicator method was used to determine apparent fat

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., Canada. Journal Series No. 399.

digestibility by pigs (Schürch *et al.*, 1952), and by pups (Lloyd and McCay, 1954). In the case of the guinea pigs, the conventional "total collection" method was used.

All feed and feces samples were extracted with anhydrous ethyl ether for a minimum period of 16 hours. As a result, the apparent digestion coefficients for fat apply only to ether extractable materials.

Results and Discussion

Effects of Chain Length. The fats and oils under consideration were divided into three categories, using saponification values as a means of differentiation. The fats and oils having saponification values over 200 were considered to contain a preponderance of short chain fatty acids, those having saponification values between 185 and 200 were considered to contain a preponderance of fatty acids of average chain length, and those having saponification values under 185 were considered to contain a preponderance of long chain fatty acids.

The average apparent digestion coefficients of the fats and oils falling into these categories, and the simple correlation coefficients between apparent fat digestibility and the mean molecular weight of the contained fatty acids are shown in table 1.

From these values, it may be seen that a highly significant inverse relationship existed between the mean molecular weight (length of chain) of the fatty acids of the various fats and oils and their apparent digestibility by early-weaned pigs and guinea pigs. No such relationship was found in the case of pups.

The first impression of these observations is that a species difference exists which is difficult to reconcile with known facts. However, in the light of the work of Holmes (1918) and of Deuel *et al.* (1949), where a significant difference was found between the digestibility of rapeseed oil by the human and the rat, it may now be logical to assume that the rat, the guinea pig and the pig all experience some difficulty in utilizing fats containing a preponderance of long chain fatty acids, while in the case of the human and the dog, such fats are almost completely utilized.

Effects of Saturation. The same fats and oils were then divided into three new categories, using iodine numbers as a means of differentiation. The fats and oils having iodine numbers under 95 were considered to contain a preponderance of saturated fatty acids, those having iodine numbers between 95 and 135 were considered to contain a preponderance of fatty acids of average saturation, and those having iodine values over 135 were considered to contain a preponderance of unsaturated fatty acids.

The average apparent digestion coefficients of the fats and oils falling into these categories, and the simple correlation coefficients between apparent fat digestibility and the degree of saturation of the fats and oils are given in table 2.

These values show that a highly significant inverse relationship existed between the iodine number of the various fats and oils and their apparent digestibility by early-weaned guinea pigs. No corresponding relationship was found in the case of pigs or pups.

In view of the general recognition that saturated fatty acids are less efficiently utilized than the unsaturated fatty acids, it appears difficult to reconcile the high digestion coefficients obtained for the saturated fats in

TABLE 1. THE RELATION BETWEEN THE MEAN MOLECULAR WEIGHT OF THE FATTY ACIDS OF VARIOUS FATS AND THEIR APPARENT DIGESTIBILITY BY YOUNG MAMMALS

Chain length	Fat or oil	Mean molecular weight of fatty acids	Average apparent digestibility of fats and oils		
			Pigs	Guinea pigs	Pups
			%	%	%
Short	Coconut Hydrogenated coconut Butter Butter (75%)+erucic acid (25%)	Less than 260 (Sap. value over 200)	94.4± 5.6	95.5± 2.6	98.2±1.2
	Lard Mixed tallow Beef tallow Butter (50%)+erucic acid (50%)	Between 260 and 285 (Sap. value between 185 and 200)	91.9± 6.2	91.7± 5.1	95.8±6.9
Medium	Cottonseed Hydrogenated cottonseed Linseed Soybean Hydrogenated soybean Corn				
Long	Fish Hydrogenated fish Butter (25%)+erucic acid (75%) Rapeseed Hydrogenated rapeseed Erucic acid	More than 285 (Sap. value under 185)	78.2±16.9	71.2±14.3	96.5±2.5
Simple correlation coefficients			r = −0.61 (highly signif.)	r = −0.55 (highly signif.)	r = −0.17 (not signif.)

this study, and particularly the highly significant inverse relationship observed for guinea pigs.

In order to evaluate the relative influence of fatty acid chain length and degree of saturation on the apparent digestibility of fat, the data were subjected to a multiple correlation and partial regression analysis. In this analysis, the apparent digestibility of fat (y) served as the dependent variable, with the mean molecular weight of the contained fatty acids (x_1) and the iodine number of the fat or oil (x_2) as the independent variables. Relevant results of this analysis are given in table 3.

First it should be noted, as indicated by the R^2 values, that 44 and 30% of the total variability in the fat digestion coefficients, for pigs and guinea

pigs respectively, was accounted for by the two independent variables. That this is meaningful is indicated by the highly significant multiple correlation coefficients (R=0.66 and R=0.55). In the case of the pups, the non-significant multiple correlation coefficient (R=0.24) is reflected in the fact that only 6% of the total variability in the fat digestion coefficients was accounted for by the two variables.

The relative beta values, lines 3 and 4, indicate the relative importance of the independent variables in establishing the numerical value of the

TABLE 2. THE RELATION BETWEEN THE DEGREE OF SATURATION OF VARIOUS FATS AND THEIR APPARENT DIGESTIBILITY BY YOUNG MAMMALS

Degree of saturation	Fat or oil	Iodine number of fat or oil	Average apparent digestibility of fats or oils		
			Pigs	Guinea pigs	Pups
			%	%	%
Saturated	Coconut Hydrogenated coconut Butter Beef tallow Mixed tallow Butter (75%)+ erucic acid (25%) Hydrogenated rapeseed Lard Hydrogenated cottonseed Butter (50%)+ erucic acid (50%) Hydrogenated fish Hydrogenated soybean Butter (25%)+ erucic acid (75%)	Under 95	92.3± 7.5	92.2± 8.6	96.6±5.9
Medium	Erucic acid Rapeseed Cottonseed Corn Fish Soybean	Between 95 and 135	84.3±16.3	86.6±13.2	97.1±1.9
Unsaturated	Linseed	Over 135	89.1	82.0± 3.7	97.5±1.3
Simple correlation coefficients:			r = −0.26 (not signif.)	r = −0.43 (highly signif.)	r = −0.02 (not signif.)

dependent variable, i.e., apparent digestibility of the fat. The relative beta values, or standard regression coefficients, show that, for pigs and guinea pigs, the greatest proportion (70 and 92% respectively) of the variability in fat digestibility accounted for is traceable to the mean molecular weight of the contained fatty acids. In the case of pups, the relative beta values were more alike in magnitude.

Lines 5 and 6 indicate the percentage of the total variability in fat digestibility traceable to mean molecular weight and iodine number respectively. (For example, Line 5 = Line 1 × Line 3 for each species, and Line 6 = Line 1 × Line 4 for each species). Thus for pigs and guinea pigs approximately 30% of the total variability was due directly to the chain

length of the contained fatty acids. For these species, degree of saturation of the fat or oil was found to exert a minor influence on fat digestibility. This was found true for guinea pigs in spite of the highly significant inverse relationship observed between iodine number and fat digestibility by simple correlation. This further emphasized the fact that, in problems of this nature, simple correlation cannot with certainty be interpreted as indicating simple cause and effect.

The small percentage of the total variability in fat digestibility by dogs which was traceable to either of the variables indicates that neither fatty acid chain length nor degree of saturation is influential in determining fat utilization by this species.

TABLE 3. INTERPRETATION OF DATA USING MULTIPLE CORRELATION AND PARTIAL REGRESSION

Line	Statistical measurement	Species		
		Pigs	Guinea pigs	Pups
1	R^2	0.44	0.30	0.06
2	R (multiple correlation coefficient)	0.66*	0.55*	0.24
3	Byx_1 (relative beta value or standard regression coefficient)	70.1%	91.9%	58.9%
4	Byx_2 (relative beta value or standard regression coefficient)	29.9%	8.1%	41.1%
5	% of total variability accounted for by mean molecular weight	31	28	4
6	% of total variability accounted for by iodine number	13	2	2

* Highly significant, P=0.01.

These results indicate that, insofar as fatty acid chain length is concerned, a real species difference exists. The dog apparently becomes allied with the human as two species that almost completely utilize fats or oils containing a large proportion of long chain fatty acids; on the other hand, the pig and the guinea pig join the rat to represent three species that utilize such fats or oils with considerably less efficiency. The fallacy of using the rat as a pilot animal in studies involving certain nutritional properties of fats or oils for humans becomes obvious.

Summary

A highly significant inverse relationship was found to exist between the mean molecular weight (length of chain) of the fatty acids of various fats and oils and their apparent digestibility by pigs and guinea pigs of suckling age. Approximately 30% of the total variability in the apparent digestibility of the fats and oils by these species was attributable to fatty acid chain length.

The relationship between the degree of saturation of the fats and oils

and their apparent digestibility by the young pigs and guinea pigs was of minor significance.

Neither mean molecular weight nor degree of saturation had any significant effect on the apparent digestibility of the fats or oils by the early-weaned pups.

A species difference with respect to ability to utilize long chain fatty acids was indicated. Apparently the dog and the human are better able to digest fats or oils containing a large proportion of long chain fatty acids than are the pig, the guinea pig and the rat.

Literature Cited

Cheng, A. L. S., M. G. Morehouse and H. J. Deuel, Jr. 1949. The effect of the level of dietary calcium and magnesium on the digestibility of fatty acids, simple triglycerides, and some natural and hydrogenated fats. J. Nutr. 37:237.

Deuel, H. J. Jr., A. L. S. Cheng and M. G. Morehouse. 1948. The digestibility of rapeseed oil in the rat. J. Nutr. 35:295.

Deuel, H. J. Jr., R. M. Johnson, C. E. Calbert, J. Gardiner and B. Thomas. 1949. Studies on the comparative nutritive value of fats. XII. The digestibility of rapeseed and cottonseed oils in human subjects. J. Nutr. 38:369.

Hoagland, R. and G. G. Snider. 1943a. Digestibility of some animal and vegetable fats. J. Nutr. 25:295.

Hoagland, R. and G. G. Snider. 1943b. Digestibility of certain higher saturated fatty acids and triglycerides. J. Nutr. 26:219.

Holmes, A. D. 1918. Digestibility of some seed oils. U. S. Dept. Agr. Bul. No. 687.

Holmes, A. D. and H. J. Deuel, Jr. 1921 Digestibility of some hydrogenated oils. Am. J. Physiol. 54:479.

Langworthy, C. F. and A. D. Holmes. 1915. Digestibility of some animal fats. U. S. Dept. Agr. Bul. No. 310.

Lloyd, L. E. and C. M. McCay. 1954. The use of chromic oxide in digestibility and balance studies with dogs. J. Nutr. 53:613.

Mattil, K. F. and J. W. Higgins. 1945. The relationship of glyceride structure to fat digestibility. 1. Synthetic glycerides of stearic and oleic acids. J. Nutr. 29:255.

Schürch, A. F., E. W. Crampton, S. R. Haskell and L. E. Lloyd. 1952. The use of chromic oxide in digestibility studies with pigs fed ad libitum in the barn. J. Animal Sci. 11:261.

Thomasson, H. J. 1955a. The biological value of oils and fats. 1. Growth and food intake on feeding with natural oils and fats. J. Nutr. 56:455.

Thomasson, H. J. 1955b. The biological value of oils and fats. III. The longevity of rats fed rapeseed oil or butterfat-containing diets. J. Nutr. 57:17.

Thomasson, H. J. 1956. The biological value of oils and fats. IV. The rate of intestinal absorption. J. Nutr. 59:343.

Thomasson, H. J. and J. Boldingh. 1955. The biological value of oils and fats. II. The growth-retarding substance in rapeseed oil. J. Nutr. 56:469.

THE DIGESTIBILITY OF RATION NUTRIENTS BY THREE- VS. SEVEN-WEEK OLD PIGS

L. E. Lloyd, E. W. Crampton and V. G. MacKay

Department of Nutrition, Macdonald College (McGill University), P.Q., Canada [1]

RELATIVELY little is known about the digestive ability of the pig prior to eight weeks of age. Recent findings at the Iowa Agricultural Experiment Station and at the University of British Columbia indicate that significant changes in digestive secretions occur prior to this age.

Lewis *et al.* (1955) have stated that the baby pig lacks fully developed proteolytic and amylolytic enzyme systems. Kitts *et al.* (1956) have shown that pancreatic amylase increases markedly with advancing age in the suckling pig, but that pancreatic lipase is of a high order at birth and remains high as growth proceeds. Bailey *et al.* (1956) found that lactase activity was of a high order from birth through the first two weeks of life, at which time a precipitous decline in activity was noted which reached minimal levels after 3–4 weeks. Sucrase and maltase activity were found to increase from negligible levels at birth to a maximum after 25 days.

The research to be described was designed therefore, to determine whether any actual changes occur in the apparent digestibility of the nutrients of a practical early-weaning ration by pigs between three and seven weeks of age.

Trial 1

Experimental

Thirty-two Yorkshire pigs, weaned at two weeks of age, were penned in groups of four (2 males and 2 females) and fed for a period of 6 weeks the dry early-weaning ration [2] of Crampton and Ness (1954). Upon reaching three and again at seven weeks of age, the apparent digestibility of certain ration nutrients was determined for each group of four pigs using the chromic oxide method of Schürch *et al.* (1952). Because the feces from each pen was composited during the collection period, each digestion coefficient obtained represented the average coefficient for 4 pigs.

The ration fractions studied included dry matter, energy, crude protein, ether extract and total carbohydrate. Feed and feces samples were analyzed for calories using the Parr peroxide bomb, for crude protein by the

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., Canada. Journal Series No. 400.

[2] Ration Composition—ground wheat, 10%; ground oat groats, 15%; skimmilk powder, 37%; soybean oilmeal, 10%; fishmeal, 10%; brewers' dried yeast, 5%; molasses, 10%; vitamin-mineral-antibiotic supplement, 3%.

TABLE 1. THE APPARENT DIGESTIBILITY OF VARIOUS NUTRIENTS OF A DRY EARLY-WEANING RATION BY PIGS OF THREE VS. SEVEN WEEKS OF AGE

	Apparent digestion coefficients									
	Dry matter		Calories *		Crude protein *		Ether extract		Total carbohydrate	
Pen No.	3 weeks	7 weeks	3 weeks	7 weeks	3 weeks	7 weeks	3 weeks	7 weeks	3 weeks	7 weeks
1 [a]	83	86	85	88	86	88	70	88	87	91
2	86	88	88	91	87	90	18	31	94	94
3	84	86	84	89	85	89	22	52	90	90
4	84	85	86	88	86	90	48	63	90	88
5	80	84	82	86	79	84	56	61	87	91
6	87	89	90	92	88	91	42	52	93	94
7	87	86	88	89	87	89	20	44	93	92
8	83	84	84	87	84	87	38	50	87	89
Mean	84.4	85.9	85.8	88.6	85.3	88.5	39.1	55.1	90.1	91.1
	±2.5	±1.8	±2.6	±2.0	±2.6	±2.2	±18.6	±16.7	±2.9	±2.3

* Difference between mean digestion coefficients for 3- vs. 7-week old pigs statistically significant at 5% level.
[a] The digestion coefficients indicated for each pen represent averages of 4 pigs.

Kjeldahl method (N $\times$ 6.25) and for ether extract by extracting with anhydrous ethyl ether for a minimum period of 16 hours.

Because of the negligible amounts of crude fiber in the ration, total carbohydrate was determined "by difference."

Chromic oxide was determined using the method of Bolin *et al.* (1952).

Results and Discussion

The average apparent digestibility of the dry matter, energy, crude protein, ether extract and total carbohydrate of the dry early-weaning pig ration by each pen of 4 pigs at three and at seven weeks of age is given in table 1.

These data show that, exclusive of total carbohydrate, in practically all cases the average digestibility of each nutrient by each group of 4 pigs was higher at seven than at three weeks of age. This trend seems significant in spite of the fact that only in the case of calories and crude protein was statistical significance reached between the mean values of all 32 pigs at the two ages.

In view of the previously mentioned findings that the baby pig lacks a fully developed amylolytic enzyme system, and that the sucrase and maltase activity increases from negligible levels at birth to a maximum after 25 days, it was surprising that a difference was not found in carbohydrate digestibility between the three- and seven-week old pigs.

The differences in protein digestibility bear out the Iowa findings that the pig, prior to 25 lb. liveweight, does not possess a fully developed proteolytic enzyme system. That protein digestion increases with age appears not to be confined to pigs. In studies with dogs, Lloyd and McCay (1955) showed that, when compared to young adult or old dogs, pups were less able to digest the crude protein in low-protein diets. In more recent work with dairy calves, Hogue *et al.* (1956) found that apparent protein digestibility was increased at 16 weeks as compared with 7 weeks of age.

The greatest numerical difference in digestibility between the three- and seven-week old pigs occurred in the ether extract fraction. The mean values of all 32 pigs at the two ages did not reach statistical significance because of the high variability within age groups. However, work with other species suggests that these differences were not merely coincidental. For example, from the studies of Holt *et al.* (1919a, 1919b), it is evident that the digestibility of fat is less efficient in infants and in young children than in older children and adults. Harrison and Sheldon (1927) have also recorded a lower digestibility of fat in children than in adults. Lloyd and McCay (1955) have shown that pups are inferior to young adult or old dogs in their ability to digest large amounts of fat. These combined observations, therefore, led to the conduct of the research reported in Trial 2.

Trial 2

Experimental

Eighty Yorkshire pigs, weaned at two weeks of age, were penned in groups of four (2 males and 2 females). These pigs were fed the same basal ration, but to which 13 different fats or oils were added individually at the 20% level (table 2).

The apparent digestibility of the same ration nutrients was determined as in Trial 1 for the pigs at three and at seven weeks of age.

Results and Discussion

After the 13 different fats or oils had been added individually to the

TABLE 2. THE APPARENT DIGESTIBILITY BY PIGS OF THREE VS. SEVEN WEEKS OF AGE OF VARIOUS NUTRIENTS OF A DRY EARLY-WEANING RATION CONTAINING DIFFERENT FATS AND OILS AT A 20% LEVEL

Fatty acid chain length	Apparent digestion coefficients for					
	Short [a]		Medium [b]		Long [c]	
Mean molecular wt.	233		277		308	
Age of pigs, weeks	3	7	3	7	3	7
Dry matter	81	87	77	84	71	82
Calories	82	91	76	87	67	86
Crude protein	86	93	85	92	85	92
Ether extract	86	96	70	89	37	78
Total carbohydrate	82	88	81	84	89	87
Mean stand. deviation	3.4	1.8	9.3	5.0	8.9	6.7

[a] Averages of six pens on butter, one on coconut oil and one on a mixture of butter 75 + erucic acid 25.

[b] Averages of one pen on lard, one on beef tallow, one on linseed oil, two on corn oil and one on a mixture of butter 50 + erucic acid 50.

[c] Averages of one pen on fish oil, one on hydrogenated fish oil, two on rapeseed oil, one on erucic acid, and one on a mixture of butter 25 + erucic acid 75.

basal ration at the 20% level and fed to early-weaned pigs, the findings reported by Lloyd and Crampton (1957) with regard to the effect of fatty acid chain length on fat digestibility by the pig became known. They reported that a highly significant inverse relationship was found between mean molecular weight (length of chain) of the fatty acids of various fats and oils and their apparent digestibility by early-weaned pigs and guinea pigs. Therefore, the rations containing the various fats or oils were grouped according to the meal molecular weight of the contained fatty acids as shown in table 2.

The average apparent digestibility of the dry matter, energy, crude protein, ether extract and total carbohydrate of each group of rations by pigs at three and at seven weeks of age is given in table 2.

As in Trial 1, and again with the exception of the total carbohydrate fraction, the average digestibility of each nutrient within each group of rations was greater by the 7- than by the 3-week old pigs. In this trial the differences were of greater magnitude and attained significance in all cases. The increased digestibility of dry matter and energy by the older pigs was a direct result of increased crude protein and ether extract digestibility by these animals.

The most pronounced differences in digestibility between the two ages studied occurred for the ether extract fraction. In addition, it was noted that there was a progressive decrease in the apparent digestibility of the fat fraction when the mean molecular weight of the fatty acids in the fats or oils increased. This, along with the interrelationship between this decrease and age of pig, is shown in table 3.

TABLE 3. INTERRELATIONSHIP BETWEEN CHAIN LENGTH AND AGE OF ANIMAL ON THE APPARENT DIGESTIBILITY OF FATS AND OILS BY EARLY WEANED PIGS

Type of fat	Mean digestibility at		Reduction in digestibility from short chain	
	3 weeks	7 weeks	3 weeks	7 weeks
	%	%	%	%
Short chain	86	96	—	—
Medium chain	70	90	19	6
Long chain	37	78	57	18

It is interesting to note from these data that, for both ages of pigs, the percentage reduction in digestibility from the short-chain fats was three times greater for the long-chain fats than for the medium-chain fats. In addition, the percentage reduction in digestibility of both the medium-chain fats and the long-chain fats from those of short-chain length was approximately three times greater for the younger than for the older animals. Therefore, aside from the fact that the apparent digestibility of the fat decreases with an increased mean molecular weight of the contained fatty acids, it was shown that this decrease was accentuated in the case of the younger pigs.

These results indicate that there is a marked change in the digestive ability of the young pig prior to 8 weeks of age, and in particular, with reference to the digestibility of fat. Since Kitts *et al.* (1956) showed that pancreatic lipase activity in pigs is of a high order at birth, it may be postulated that the increase in digestibility of fat with advancing age is due to an increase in bile secretion, resulting in an increased absorptive capacity for fat.

Summary

The average apparent digestibility of the dry matter, energy, crude protein and ether extract of a dry early-weaning pig ration was higher for 7- than for 3-week old pigs. The greatest difference occurred in the ether extract fraction. Age of pig had no significant effect on carbohydrate digestibility.

A progressive decrease in the apparent digestibility of fat was noted when the mean molecular weight of the contained fatty acids increased. This decrease was accentuated in the case of the younger pigs. The results indicated that a marked change in the digestive ability of the young pig takes place prior to 8 weeks of age.

Literature Cited

Bailey, C. B., W. D. Kitts and A. J. Wood. 1956. The development of the digestive enzyme system of the pig during its pre-weaning phase of growth. B. Intestinal lactase, sucrase and maltase. Canadian J. Agr. Sci. 36:51.

Bolin, D. W., R. P. King and E. W. Klosterman. 1952. A simplified method for the determination of chromic oxide (Cr_2O_3) when used as an index substance. Science 116:634.

Crampton, E. W. and O. M. Ness. 1954. A meal mixture suitable as the entire ration to be self-fed dry to pigs weaned at ten days of age. J. Animal Sci. 13:357.

Harrison, G. A. and W. P. H. Sheldon. 1927. Faecal fat analysis in children. Arch. Disease in Childhood 2:338.

Hogue, D. E., R. G. Warner, C. H. Griffin and J. K. Loosli. 1956. Digestion coefficients and nitrogen retention of young dairy calves as affected by antibiotics and advancing age. J. Animal Sci. 15:788.

Holt, L. E., A. M. Courtney and H. L. Fales. 1919a. Study of the fat metabolism of infants and young children: Fat in the stools of breastfed infants. Am. J. Diseases Children 17:241.

Holt, L. E., A. M. Courtney and H. L. Fales. 1919b. Fat metabolism of infants and young children: Fat in the stools of children on a mixed diet. Am. J. Diseases Children 18:107.

Kitts, W. D., C. B. Bailey and A. J. Wood. 1956. The development of the digestive enzyme system of the pig during its pre-weaning phase of growth. A. Pancreatic amylase and lipase. Canadian J. Agr. Sci. 36:45.

Lewis, C. J., D. V. Catron, C. H. Liu, V. C. Speer and G. C. Ashton. 1955. Enzyme supplementation of baby pig diets. J. Agr. and Food Chem. 3:1047

Lloyd, L. E. and C. M. McCay. 1955. The utilization of nutrients by dogs of different ages, J. Gerontol. 10:182.

Lloyd, L. E. and E. W. Crampton. 1957. The relation between certain characteristics of fats and oils and their apparent digestibility by young pigs, young guinea pigs and pups. J. Animal Sci. 16:377.

Schürch, A. F., E. W. Crampton, S. R. Haskell and L. E. Lloyd. 1952. The use of chromic oxide in digestibility studies with pigs fed ad libitum in the barn. J. Animal Sci. 11:261.

PROTEIN REQUIREMENTS FOR PIGS WEANED AT TWO WEEKS OF AGE

A. H. JENSEN, D. E. BECKER, H. W. NORTON AND S. W. TERRILL [1]
Illinois Agricultural Experiment Station, Urbana

SEVERAL investigators have reported studies of protein levels for the young pig fed liquid diets. Reber *et al.* (1953) employed a vitamin-extracted casein as the sole source of protein and reported that a diet containing 41% protein produced maximum weight gains and feed efficiency in pigs from 12 to 24 days of age. As the pigs approached 8 weeks of age 20% protein appeared to be as efficient as higher levels. Using a crude casein and an isolated soybean protein supplemented with methionine, Sewell *et al.* (1953) found that 16 or 20% protein produced slow growth in baby pigs from 2 to 30 days of age. Twenty-four and 28% protein levels gave relatively satisfactory results, but fastest growth and highest feed efficiency resulted when a level of 32% protein was fed. Becker *et al.* (1954) made extensive studies with the young pig fed dried skim milk as the source of protein. These workers concluded that 22% and 12% protein levels were minimum requirements for the periods from 1 to 4 weeks and 5 to 9 weeks of age, respectively, and, after analysis of the diets used, calculated estimates of amino acid requirements for these periods.

Crampton and Ness (1954) reported studies on 10-day-old pigs weaned directly to dry diets. Thirty percent protein in the diet produced significantly faster gains than 26% protein, the former increasing feed efficiency 15% over the latter. With pigs weaned at one week of age directly to dry diets, Peo *et al.* (1954) fed protein levels of 15, 20, 25 and 30%. Twenty percent protein produced gains and feed efficiency comparable to the higher levels, while 15% produced slower and less efficient growth.

The investigations reported herein were conducted to determine the minimum protein requirement for pigs weaned at approximately 2 weeks of age and fed dry diets until 8 weeks of age. The primary source of protein was a mixture of 2.16 parts corn to 1 part crude casein.

Experimental

Sixty pigs weaned at 12 to 16 days of age were used in each of three experiments. Pigs from Yorkshire and crossbred sows were used in Experiments 1 and 3, and pigs from Yorkshire gilts were used in Experiment 2.

[1] The authors wish to acknowledge Moorman Manufacturing Company, Qunicy, Illinois; Commercial Solvents Corporation, Terre Haute, Indiana; Merck and Company, Rahway, New Jersey; American Cyanamid Company, Pearl River, New York and Midwest Dried Milk Company, Dundee, Illinois, for funds and products which made this study possible.

When 30 pigs became available a replicate was started on test. Litter-mate outcome groups of six pigs each were formed on the basis of weight and condition at weaning. Pigs within outcome groups were randomly assigned to protein levels within each of the two replicates per experiment.

Each lot of five pigs was confined to a 4′ x 10′ concrete floor pen. An automatic waterer and a self-feeder were provided for each pen. Supplementary heat was provided by a 250-watt heat lamp suspended above a 30″-square wooden overlay in one corner of the pen.

TABLE 1. COMPOSITION OF DIETS USED

Ingredients	Percent
Ground yellow corn [a]) [b]	
Crude casein [a])	86.8
Cerelose	
Dried whey	2.5
Condensed fish solubles	2.5
Dried brewer's yeast	2.0
Wheat germ oil	2.0
Di-calcium phosphate) [c]	3.5
Ground limestone)	
Iodized salt	0.5
Mineral mix #1 [d]	0.2
Vitamin-antibiotic mix [e]	++

[a] This mixture contained 68.4% corn and 31.6% crude casein.

[b] Protein levels were obtained by substituting the corn and casein mixture for cerelose.

[c] These ingredients added to provide 1.1% calcium and 0.8% phosphorus in the total ration.

[d] Composition of mineral mixture in percent: $MgCO_3$, 68.6; $FeSO_4$, 20.0; $MnSO_4 \cdot H_2O$, 6.0; $CoCl_2 \cdot 6H_2O$, 2.0; $CuSO_4$, 2.0; NaF, 0.4, $ZnCO_3$, 0.8; and KI 0.2.

[e] Contributes per pound of ration: Vitamin K 0.85 mg; ascorbic acid, 13.6 mg; thiamine-HCl, 2.2 mg; riboflavin 4.5, mg; pyridoxine-HCl, 2.2 mg; calcium pantothenate, 25 mg; inositol, 85 mg; choline chloride, 682 mg; PABA, 8.8 mg; biotin, 0.03 mg; niacin, 14 mg; folic acid, 0.18 mg; vitamin B_{12}, 0.013 mg; chlortetracycline HCl, 30 mg; procaine penicillin, 30 mg; vitamin A, 4320 I. U. and Vitamin D_2 540 I. U.

The ingredients used in the diets fed are shown in table 1. This corn-casein diet had previously given results equal to those obtained with diets containing 50% dried skim milk when fed to pigs weaned at two weeks of age. Further, its texture was favorable to free-flow in the self-feeder employed.

Individual pig gains and lot feed consumption were determined every 14th day. The data were analyzed according to Snedecor (1946).

Results

The results of Experiments 1 and 2 are shown in table 2.

In Experiment 1 the ration containing 14.4% crude protein was inadequate for optimum performance by the two-week-old pig. However, the 16.6% crude protein ration produced gains comparable to those realized

TABLE 2. RESULTS OF EXPERIMENTS 1 AND 2

Protein level [a]	14.4	16.6	19.4	22.0	24.8	27.6	Av.
No. of pigs	20	20	20	20	20	20	
Av. initial wt., lb.							
Exp. 1	7.8	7.8	7.4	7.7	7.9	7.7	7.7
Exp. 2	8.0	7.8	7.9	7.9	7.9	7.8	7.9
Av.	7.9	7.8	7.6	7.8	7.9	7.7	
Av. final wt., lb.							
Exp. 1	36.5	43.8	41.4	40.5	42.1	42.6	41.1
Exp. 2	36.5	38.2	39.3	35.9	39.7	41.0	38.4
Av.	36.5	41.0	40.3	38.2	40.9	41.8	
Av. daily gain, lb.							
Exp. 1	0.68	0.85	0.81	0.78	0.81	0.83	0.79
Exp. 2	0.67	0.71	0.74	0.65	0.74	0.78	0.72
Av.	0.67	0.78	0.78	0.72	0.78	0.80	
Av. feed per lb. of gain, lb.							
Exp. 1	2.13	2.01	1.96	1.85	1.80	1.71	1.91
Exp. 2	2.04	1.83	1.92	1.78	1.82	1.78	1.86
Av.	2.08	1.92	1.94	1.81	1.81	1.75	

[a] Determined by chemical analysis (N x 6.25).

on the higher protein level diets fed. A similar, though less clear-cut, trend in rate-of-gain response to protein levels was obtained in the second experiment. There is no apparent explanation for the lowest growth rate being produced by the pigs receiving a 22% protein ration.

In these two experiments the feed required for each pound of gain was 2.13 and 2.04 lb. on the lowest protein level and 1.71 and 1.78 lb. on the highest protein level, respectively. Whereas, in Experiment 1 there was a definite decrease in the quantity of feed required per pound of gain for each increment in protein level fed, in Experiment 2 the variation between

TABLE 3. ANALYSIS OF VARIANCE OF AVERAGE DAILY GAINS FOR EXPERIMENTS 1 AND 2

	Average daily gains		Feed per lb. of gain	
Source	d.f.	Mean square	d.f.	Mean square
Experiment (E)	1	.1703	1	.0145
Protein level (P)	5	.0456**	5	.0598*
E x P	5	.0115	5	.0070
Replicates within Exp. (R)	2	.0019	2	.0666*
R x T	10	.0129	10	.0159
Outcome groups (G)	16	.0542**		
G x T	79 [a]	.0116		
Total	118		23	

* P=<0.05.
** P=<0.01.
[a] One degree of freedom lost because of a missing value.

TABLE 4. SUMMARY OF RESULTS OF EXPERIMENT 3

Protein level [a]	10	13	16	22	28	31.8
No. of pigs	10	10	10	10	10	10
Av. initial wt., lb.	9.9	10.0	9.9	10.1	10.1	10.2
Av. final wt., lb.	29.4	31.5	40.8	41.1	41.9	43.7
Av. daily gain, lb.	0.46	0.51	0.73	0.74	0.76	0.80
Av. feed per lb. of gain, lb.	2.97	2.63	2.25	2.22	2.07	2.02

[a] Calculated values.

protein levels was greater and the 22% protein ration produced as efficient gains as did the highest level.

Analysis of variance of the pooled data for Experiments 1 and 2 is shown in table 3. These analyses show that protein levels significantly affected both average daily gains and feed required per pound of gain, $P<0.01$ and $P<0.05$, respectively.

Since the data from the first two experiments evidenced a trend of increasing feed efficiency with each increment in protein fed, Experiment 3 was designed to study a wider range of protein levels and accumulate feed data from which an estimate of the minimum protein requirement could be made.

In table 4 it can be seen that the protein levels fed varied from 10 to approximately 32%, and the analysis of variance, table 5, shows that protein level significantly affected the average daily gains ($P<0.001$) and feed efficiency ($P<0.01$). Average daily gains increased markedly up to the 16% protein level, while the total increase in rate of gain above this level was only 0.07 lb. As would be expected, the amount of feed per unit of gain decreased sharply when the protein level was increased up to 16%. No further advantage was realized with 22% protein, but with 28 and 31.8% protein levels increased feed efficiency was obtained.

TABLE 5. ANALYSIS OF VARIANCE OF EXPERIMENT 3

	Average daily gains		Feed per lb. of gain	
Source	d.f.	Mean square	d.f.	Mean square
Protein levels (P)	5	.2026***	5	.2710**
Replicates (R)	1	.1904	1	.0004
P x R	5	.0118	5	.0108
Outcome groups	8	.0440**		
Remainder	40	.0125		
Total	59		11	

** P=<0.01. *** P=<0.001.

The method of least squares was used to fit bent lines to the feed efficiency data for all three experiments, as shown in figure 1. The three lines were found to have the same slope and to bend at the same point, and were found to fit the data adequately. Thus they provide a pooled estimate of 17.35±0.52% as the minimum level of protein required by the young pig under these conditions. This agrees very well with the gain data, which indicated 16 and 16.6% protein to be adequate.

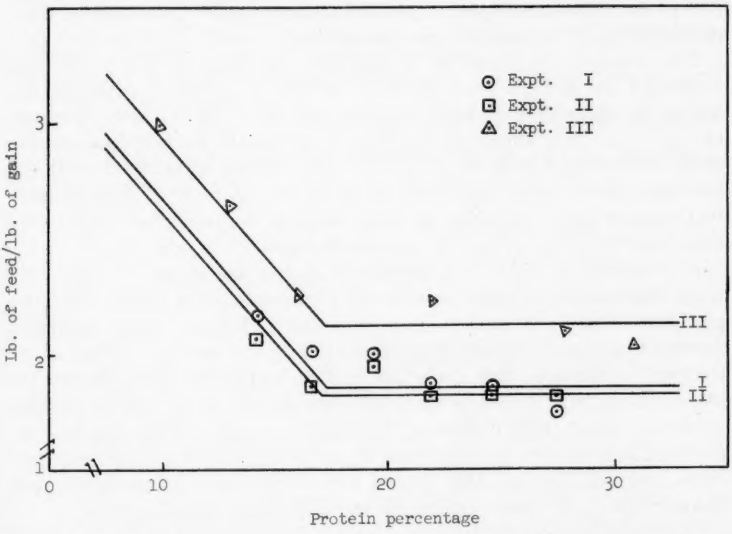

Figure 1. Feed efficiency data for three experiments, with bent lines (fitted by least squares) differing only in level attained.

Discussion

Results reported here show that pigs weaned at 14 days of age and fed a corn and crude casein dry diet until 56 days of age require approximately 17% protein in the ration. This estimate of the requirement is based on the facts that (1) 16 and 16.6% protein levels produced average daily gains comparable to the higher levels fed, and (2) the method of least squares applied to the feed data found the optimum response at 17.35%±0.52 protein. This level is lower than those previously suggested. Sewell *et al.* (1953) using liquid diets for pigs from 2 to 30 days of age, reported increased performance with Alpha-protein + methionine and casein diets up to 28 and 30% protein levels, respectively. Reber *et al.* (1953) fed liquid diets and observed maximum responses with 41% and 20% protein levels for pigs from 12 to 24 days of age and approaching 8

weeks of age, respectively. On the other hand, Becker *et al.* (1954) placed the protein requirements at 22 and 12%, respectively, for suckling pigs from 1 to 4 weeks and 5 to 9 weeks of age, claiming these lower levels were adequate when dried skim milk served as the sole source of protein.

The estimated protein requirement, approximately 17%, reported herein is considerably below the two levels reported by Crampton and Ness (1954). In their studies 30% protein rations gave significantly faster gains than 26% protein rations. It is also lower than the 20% protein level which Peo *et al.* (1954) reported as producing best overall performance when compared to 15, 25 and 30% protein rations.

The apparent discrepancies in minimum requirements when dry diets were used are perhaps due in part to differences between the rations employed in the various studies. Crampton and Ness (1954) used diets containing 20 to 37% dried skim milk and up to 52% cereal products. Peo *et al.* (1954) used a 50-50 mixture of 50% solvent soybean oil meal and low-heat, spray dried skim milk as the source of protein. It is possible that balance and availability of amino acids in the corn plus crude casein diets were superior to those in the above studies.

It is interesting that 17% protein is an average of the 22 and 12% levels Becker *et al.* (1954) reported as minimum protein requirements for pigs 1 to 4 and 5 to 9 weeks of age, respectively. Whereas, they reported a marked decrease in protein requirement during the normal suckling stage, the pigs on the corn plus crude casein diets employed here indicated the same protein requirement in each two-week period of the test. It is noteworthy, however, that it took up to 5 days for some of the pigs to completely adapt to the dry diets and to evidence positive growth response. Thus, the pigs had possibly passed the marked decreasing requirement phase of the 1- to 4-week period discussed by Becker *et al.* (1954).

Summary

Three experiments involving 180 baby pigs were conducted to study the response to different levels of protein by pigs weaned at 2 weeks of age and fed until 8 weeks of age. The primary source of protein was a mixture of 2.16 parts ground corn and 1 part crude casein.

Ten protein levels ranging from 10 to 31.8% were studied. Growth rate and feed efficiency were significantly affected by protein level fed. Sixteen percent protein in Experiment 3 and 16.6% protein in Experiments 1 and 2 produced rates of gain about equal to those obtained on higher protein levels. Feed required per pound of gain decreased markedly as protein level increased up to the 16 and 16.6% levels. Above these levels, there appeared to be a trend toward further increase in feed efficiency. However, the method of least squares applied to the feed data from all three experiments resulted in an estimated minimum protein requirement of $17.35 \pm 0.52\%$.

When a mixture of 2.16 parts corn and 1 part crude casein served as the primary protein source in dry diets for two-week-old, weaned pigs, the minimum protein requirement appeared to be approximately 17%.

Literature Cited

Becker, D. E., D. E. Ullrey and S. W. Terrill. 1954. Protein and amino acid intakes for optimum growth rate in the young pig. J. Animal Sci. 13:346.

Crampton, E. W. and O. M. Ness. 1954. A meal mixture suitable as the entire ration to be self-fed dry to pigs weaned at ten days of age. J. Animal Sci. 13:357.

Peo, E. R., Jr., G. C. Ashton, V. C. Speer and D. V. Catron. 1954. Protein and fat requirements of baby pigs. J. Animal Sci. 13:995.

Reber, E. F., C. K. Whitehair and R. W. MacVicar. 1953. The effect of level of protein fed baby pigs. J. Nutr. 50:451.

Sewell, R. F., B. E. Sheffy, R. G. Eggert and J. K. Loosli. 1953. Studies on the protein requirement of suckling pigs. J. Animal Sci. 12:597.

Snedecor, G. W. 1946. Statistical Methods. 4th Edition. Iowa State College Press, Ames, Iowa.

EFFECTS OF DIETARY PANTOTHENIC ACID LEVELS ON THE WEANLING PIG[1, 2]

C. E. BARNHART,[3] D. V. CATRON, G. C. ASHTON [4] AND L. Y. QUINN [5]
Iowa Agricultural Experiment Station [6]

EXTENSIVE research has shown that pantothenic acid is necessary in the rations of pigs if physiological functions are to proceed normally. Pantothenic acid deficiency symptoms have been demonstrated and reported in pigs by Wintrobe *et al.* (1938, 1939, 1940, 1942, and 1943), Follis and Wintrobe (1945), Ellis and Madsen (1941) and Ellis *et al.* (1943).

Silber (1944) reported that although significant decreases were found in the pantothenic acid levels of blood, urine and feces of several depleted dogs, the possibility of revealing a deficiency state by any single determination seems slight.

McMillen and associates (1949) reported that niacin, pantothenic acid and riboflavin are stored for only 2 to 4 weeks in weanling pigs.

Nelson *et al.* (1947) found that urinary excretion of pantothenic acid in rats was affected by the protein level fed. Pearson and Schmidt (1948) stated that the amount of pantothenic acid excreted in the urine by ponies was definitely influenced by the amount of the vitamin ingested. Wiese *et al.* (1951) reported that urinary pantothenic acid excretion in the pig increased with dietary pantothenic acid levels.

Elvehjem *et al.* (1941) reported that balance studies showed that the feces from rats always contain a significant amount of pantothenic acid and this amount is independent of the intake. Henderson *et al.* (1942) reported that 50 to 60% of the pantothenic acid added to rat diets was recovered in the feces. They stated the amount of the vitamin excreted in the feces was almost independent of the diet. Rats with a severe deficiency excreted as much pantothenic acid per gram of feces as rats on an adequate intake. These authors suggested that most of the fecal pantothenic acid was from bacterial synthesis.

Axelrod *et al.* (1947) reported that when either pyridoxine, riboflavin or pantothenic acid was withdrawn from the ration of rats, the animals' antibody response to human red cells was decreased.

The object of this study was to evaluate the relation between rate of

[1] Journal paper No. J-2946 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 930.

[2] From a portion of a thesis presented by C. E. Barnhart to the Graduate College, Iowa State College, in partial fulfillment of the requirements for the Ph.D. degree, 1954.

[3] Present address: Animal Husbandry Department, University of Kentucky, Lexington.

[4] Department of Physics, Ontario Agricultural College, Guelph.

[5] Department of Bacteriology.

[6] Animal Husbandry Department.

Acknowledgment is made to Miss Helen Maddock of American Cynamid Co., New York, N. Y., to Dr. H. O. Hartley, Department of Statistics, and to Mr. Donald Quinn, Swine Nutrition Research Farm Superintendent and his associates for their assistance.

gain, feed efficiency, certain blood constituents, and urinary excretion of pantothenic acid of growing pigs fed a purified ration supplemented with six levels of pantothenic acid.

Experimental Procedure

Animals and Rations

Forty-eight cross-bred pigs which had been raised on sows fed adequate rations throughout gestation were used in this study. The pigs were removed from their dams when about 35 days old and were given a corn-soybean-oil-meal ration containing 12% protein and low in all B-vitamins for three weeks. This period included 12 days in the experimental environment. Four pigs, two barrows and two sows, were allotted to each of 12 ration treatments at random according to weight outcome groups within each sex. Each pig was fed and watered *ad libitum* while confined continuously in a wire-floored crate which was hosed down three times daily to help prevent coprophagy.

Because of certain limitations in metabolism equipment the barrow pigs were confined in one house with one type of equipment and the sow pigs in a second house with another type of metabolism facilities. This was not considered a serious drawback in the experimental design since sex X nutrient interaction has not been a common finding in swine feeding trials. Any effects due to different houses and equipment can not be separated from the sex effect but constitute a part of the variation associated with all sex contrasts.

Six levels of pantothenic acid, (2, 3, 4, 5, 6, or 7 mg. per lb. of ration), with or without chlortetracycline (Aureomycin HCl), were used in a 2 x 6 factorial arrangement in a randomized block design. These rations were fed to the pigs from a weight of about 25 to 100 lb. The composition of the purified ration is given in table 1. When chlortetracycline was fed, it was added at the rate of 5 mg. per lb. of ration.

Urine Collection and Analysis

The total urine excreted in a 48-hour period was collected from each pig immediately prior to feeding the experimental ration and again when each pig attained a weight of 75 lb.

The urine from each pig was used in an individual parallel-line-assay experiment to construct a graded dosage-response curve (Bliss 1951; Finney, 1952) for every pig. The concentration of calcium pantothenate in the urine was assayed microbiologically against a standard of known calcium pantothenate concentration using *Lactobacillus arabinosus* 17–5 ATCC 8014 as the assay organism. Growth in the assay tube was determined turbidimetrically after 24 hours of incubation in a water bath at 37° C. Both the urine and the standard solution levels were at ratios of 1, 2 and 4 which gave equidistant logarithmic concentration scales.

TABLE 1. COMPOSITION OF THE BASAL RATION

Ingredients	Percent
Pearl starch	37.50
Dextrose	30.00
Drackett C-1 Assay Protein	19.50
Woodflock	2.00
Corn oil	4.00
Trace mineral mix No. 594 [a]	3.00
Dicalcium phosphate	1.80
Vitamin premix No. 594 [b]	2.00
98% DL-methionine	0.20
Total	100.00

[a] Contributed the following percent of ration: Mn, 0.01; Fe, 0.036; Cu, 0.00076; Co, 0.0003; Zn, 0.003; Mg, 0.05; K, 0.40; NaCl, 0.51; I, 0.00004.

[b] Contributed the following per lb. of ration: Alpha tocopheryl acetate, 3 mg.; biotin, 0.2 mg.; ascorbic acid, 50 mg.; choline chloride, 450 mg.; pantothenic acid, 1.63 mg.; folic acid, 0.5 mg.; niacin, 14.5 mg.; riboflavin, 1.5 mg.; thiamin HCl, 3 mg.; pyridoxine, 1 mg.; vitamin K, 1 mg.; para-amino-benzoic acid, 0.5 mg.; inositol, 200 mg.; vitamin B_{12}, 5 mcg.; vitamin A, 2525 I.U.; vitamin D, 400 I.U.

On the assumption that the variation in calcium pantothenate concentration in the urine would probably be much greater between samples from different pigs than between samples from the same pig, only one tube of urine was assayed at each of the three levels of urine used for each pig. The elimination of duplicate tubes permitted handling the urine samples from all 48 pigs and the samples for the standard solution in a single assay operation.

TABLE 2. SAMPLE ANALYSIS OF VARIANCE PLAN MEAN SQUARES FOR CALCIUM PANTOTHENATE URINARY EXCRETION DIFFERENCES

Source of variation	Degrees of freedom	Mean squares
Replication	1	21.09
Sex (A)	1	52.92[a]
Antibiotic (B)	1	2.32
Level of Vitamin (C)	5	426.36
Linear regression component	1	2053.57[b]
Quadratic regression component	1	36.05
Cubic regression component	1	30.67
Remainder	2	5.76
A x B	1	43.43
A x C	5	30.83
B x C	5	17.23
A x B x C	5	21.91
Experimental error	23	21.42
Total	47	.....

[a] Mean square significant at P=0.05 or less.

[b] Linear regression component significant at P = 0.05 or less.

Following a suggestion by Finney (1952, p. 110), the growth responses obtained in the assay tubes for the three urine levels for each pig were "matched" to three approximately equal growth responses obtained from the standard solution to avoid as far as possible errors due to curvature in the log—concentration—response relationship. In this manner calcium pantothenate potencies for the initial and final excretion periods were determined. The difference in potency between the two periods was taken as the measure of the ration effect in the individual pig. This difference is the experimental unit in the analysis of variance for evaluating the significance of ration effects on urine calcium pantothenate concentration. The analysis of variance scheme used for the response criteria of this experiment is indicated in table 2.

TABLE 3. SUMMARIES OF 48-HOUR URINARY CALCIUM PANTOTHENATE EXCRETION PER PIG

	Calcium pantothenate excreted in 48 hours								
	75 lb. pigs			25 lb. pigs			Difference		
Pantothenic acid levels	No Ab.[a]	5 mg. Ab.	Av.	No Ab.	5 mg. Ab.	Av.	No Ab.	5 mg. Ab.	Av.
mg. per lb.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
2	5.0	3.4	4.2	0.3	0.3	0.3	4.7	3.1	3.9
3	5.8	6.0	5.9	0.5	0.6	0.6	5.3	5.4	5.4
4	7.4	8.8	8.1	0.8	0.6	0.7	6.6	8.2	7.4
5	16.7	11.4	14.1	0.4	0.2	0.3	16.3	11.2	13.8
6	16.8	20.3	18.5	0.3	0.3	0.3	16.5	20.0	18.2
7	22.7	21.5	22.1	0.5	0.3	0.4	22.2	21.2	21.7
Av.	12.4	11.9	...	0.46	0.38	...	11.9	11.5	...

[a] Ab., Antibiotic.

Supplementary Response Criteria

Records were taken on rate of gain, feed intake and feed efficiency. Hemoglobin, red and white blood cells, hematocrit and clotting time were determined when the pigs weighed 75 lb.

Results and Discussion

Urinary Excretion of Calcium Pantothenate

Summaries of the 48-hour urinary excretions of calcium pantothenate per pig for the 25 and the 75 lb. pigs, and the differences between these quantities, are presented in table 3. While on the average the differences in the 48-hour excretions for the antibiotic-fed pigs were lower than those for the non-antibiotic-fed pigs, they were not large enough to give confidence at the 5 percent probability level that they resulted from the use of the antibiotic. There was, however, a marked increase in calcium

pantothenate excretion as the level of the vitamin in the ration was increased.

A formal analysis of the complete regression for the urinary excretion values (table 2) into linear, quadratic and cubic components gave a highly significant linear component and noticeable but non-significant quadratic and cubic components. However, the representation of this excretion curve by a single polynomial might be a mathematical artifice since any threshold value beyond which the body can not completely absorb the vitamin is likely to cause a break in the relationship between vitamin intake and urinary excretion near the threshold level.

The Subcommittee on Swine Nutrition, N.R.C. (1953), allowance of pantothenic acid per 50 lb. pig is 4.5 mg. per lb. of ration. The calcium pantothenate excretion curve in figure 1 does indeed show a slight break

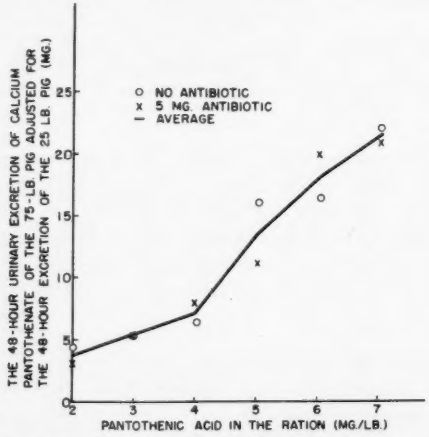

Fig. 1. Relation between level of pantothenic acid intake and urinary excretion of calcium pantothenate.

in this region. This point was put to a statistical test by contrasting the linear regressions fitted separately to the vitamin levels of 2, 3 and 4 versus 5, 6 and 7 mg. per lb. of feed. The difference between the two linear regressions with slopes of 1.78 and 3.96 gave a non-significant value for t, the lack of significance being largely due to a considerable pig-to-pig variability which is not evident in the averages (8 pigs per group) plotted as the solid line in figure 1.

Growth and Feed Responses

Summaries of rate of gain, feed consumption, and feed efficiency data are presented in table 4. The small average differences noted between the non-antibiotic and antibiotic groups were too small to be significant at

P=0.05 except for feed efficiency. In none of the three responses was any trend with level of vitamin in the ration apparent. The average growth rate and feed efficiency were quite acceptable for growing pigs from 25 to 75 lb. in weight. None of the usually accepted symptoms of pantothenic acid deficiency was observed in any of the pigs.

Response Criteria in the Blood

Average values per pig for the several blood constituents considered in this study are contained in table 5. None of the contrast differences observed was large enough to be statistically significant. The lack of differences

TABLE 4. SUMMARIES OF AVERAGE DAILY GAIN, DAILY FEED AND FEED PER LB. OF GAIN

	Av. daily gain			Av. daily feed			Feed per lb. gain		
Pantothenic acid levels	No Ab.[a]	5 mg. Ab.	Av.	No Ab.	5 mg. Ab.	Av.	No Ab.	5 mg. Ab.	Av.
mg. per lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.
2	1.18	1.32	1.25	2.88	2.92	2.90	2.44	2.21	2.32
3	1.31	1.30	1.30	3.35	3.07	3.21	2.54	2.37	2.46
4	1.18	1.30	1.24	3.01	3.10	3.05	2.54	2.38	2.46
5	1.22	1.37	1.29	3.03	3.24	3.13	2.48	2.35	2.41
6	1.34	1.31	1.33	3.26	3.02	3.14	2.41	2.30	2.35
7	1.28	1.29	1.29	3.05	2.95	3.00	2.38	2.29	2.33
Av.	1.25	1.31	...	3.10	3.05	...	2.46 *	2.32	...

[a] Ab., Antibiotic.
* Effect of antibiotic statistically significant at P = 0.05 or less.

resulting from antibiotic feeding for red blood cell counts, hemoglobin determinations and hematocrits is in agreement with the findings of Squibb *et al.* (1953). None of the blood abnormalities reported by Wintrobe *et al.* (1939, 1943) as associated with a pantothenic acid deficiency in pigs was observed here.

Summary

Forty-eight cross-bred pigs were allotted according to weight outcome groups within each sex to 12 different ration treatments to study the pantothenic acid requirement of weanling pigs. A purified ration with levels of 2, 3, 4, 5, 6 or 7 mg. of pantothenic acid with or without chlortetracycline was fed. The pigs were confined to wire-floored individual crates and self-fed from weaning until they weighed 100 lb.

There was no significant difference in the rate of gain, daily feed consumed, or feed required per lb. of gain between the pigs being fed the different ration treatments. Neither was there any significant difference in hemoglobin, red blood cell count, white blood cell count, differential white blood cell count, hematocrit or clotting time between the pigs on the 12

TABLE 5. SUMMARIES OF BLOOD DATA

Pantothenic acid levels	Hemoglobin			Red cell count			White cell count			Hematocrit, %			Clotting time		
	No Ab.[a]	5 mg. Ab.	Av.	No Ab.	5 mg. Ab.	Av.	No Ab.	5 mg. Ab.	Av.	No Ab.	5 mg. Ab.	Av.	No Ab.	5 mg. Ab.	Av.
mg. per lb.	gm. per 100 ml.			1000 per cu. mm.			Per cu. mm.						Seconds		
2	12	13	12	6,860	7,323	7,092	16,200	15,550	15,875	38	40	39	19	30	24
3	13	14	14	6,958	7,000	6,979	12,850	22,125	17,488	39	40	40	26	38	32
4	13	13	13	7,273	7,263	7,268	21,950	20,775	21,362	37	38	38	41	23	32
5	13	13	13	7,093	7,550	7,322	17,500	18,525	18,012	39	44	41	26	26	26
6	13	12	12	7,483	7,183	7,333	19,525	20,950	20,238	41	38	40	26	53	39
7	12	13	12	7,158	7,213	7,186	16,950	18,775	17,862	37	39	38	30	45	38
Av.	12	13		7,138	7,255		17,496	19,450		38	40		28	36	

[a] Ab., Antibiotic.

ration treatments. The amount of calcium pantothenate excreted in the urine when the pigs weighed 75 lb. was found to be closely related to the levels of panothenic acid the pigs were receiving in their rations.

Literature Cited

Axelrod, A. R., B. B. Carter, R. H. McCoy and R. Geisinger, 1947. Circulating antibodies in vitamin deficiency states. I. Pyridoxine, riboflavin, and pantothenic acid deficiencies. Proc. Soc. Exp. Biol. Med. 66:137.

Bliss, C. I. 1951. Statistical methods in vitamin research. Vitamin methods by Paul Gyorgy. Academic Press, Inc., New York.

Ellis, N. R. and L. L. Madsen. 1941. Relation of diet of swine to development of locomotor incoordination resulting from nerve degeneration. J. Agr. Res. 62:303.

Ellis, N. R., L. L. Madsen and C. O. Miller. 1943. Pantothenic acid and pyridoxine as factors in the occurrence of locomotor incoordination in swine. J. Animal Sci. 2:365.

Elvehjem, C. A., L. M. Henderson, S. Black and E. Nielsen. 1941. Synthetic calcium pantothenate in the nutrition of the rat. J. Biol. Chem. 140:36.

Finney, D. J. 1952. Statistical method in biological assay. Hafner Publishing Co., New York, N. Y.

Follis, R. H., Jr. and M. M. Wintrobe. 1945. A comparison of the effects of pyridoxine and pantothenic acid deficiencies on the nervous tissue of swine. J. Exp. Med. 81:539.

Henderson, L. M., J. M. McIntire, H. A. Wainsman and C. A. Elvehjem. 1942. Pantothenic acid in the nutrition of the rat. J. Nutr. 23:47.

McMillen, W. N., R. W. Luecke and F. Thorp, Jr. 1949. B-vitamins for weanling pigs. Mich. Agr. Exp. Sta. Quart. Bul. 32:191.

Nelson, M. M., F. Van Nouhuys and H. M. Evans. 1947. The sparing action of protein on pantothenic acid requirements of the rat. II. Urinary and fecal excretion of pantothenic acid. J. Nutr. 34:189.

Pearson, P. B. and H. Schmidt. 1948. Pantothenic acid studies with the horse. J. Animal Sci. 7:78.

Silber, R. H. 1944. Studies of pantothenic acid deficiency in dogs. J. Nutr. 27:425.

Squibb, R. L., Eugenio Salazar, Miguel Guzman and Nevin S. Scrimshaw. 1953. Effect of aureomycin and vitamins on growth and blood constituents of pigs fed corn and banana rations. J. Animal Sci. 12:297.

Subcommittee on Swine Nutrition. 1953. Nutrient requirements for domestic animals. Number II. Nutrient requirements for swine. National Academy of Sciences—National Research Council. Pub. 295.

Wiese, A. C., W. P. Lehrer, Jr., P. R. Moore, O. F. Pahnish and W. V. Hartwell. 1951. Pantothenic acid deficiency in baby pigs. J. Animal Sci. 10:80.

Wintrobe, M. M., J. L. Miller, Jr. and H. Lisco. 1940. The relation of diet to the occurrence of atoxia and degeneration in the nervous system of pigs. Johns Hopkins Hosp. Bul. 67:377.

Wintrobe, M. M., M. H. Miller, R. H. Follis, Jr., H. J. Stein, C. Mushatt and S. Humphreys. 1942. Sensory neuron degeneration in pigs. IV. Protection afforded by calcium pantothenate and pyridoxine. J. Nutr. 24:345.

Wintrobe, M. M., D. L. Mitchell and L. C. Kolb. 1938. Sensory neuron degeneration in vitamin deficiency. J. Expt. Med. 68:207.

Wintrobe, M. M., M. Samter and H. Lisco. 1939. Morphologic changes in the blood of pigs associated with deficiency of water-soluble vitamins and other substances contained in yeast. Johns Hopkins Hosp. Bul. 64:399.

Wintrobe, M. M., R. H. Follis, Jr., R. Alcayaga, M. Paulson and S. Humphreys. 1943. Pantothenic acid deficiency in swine with particular references to the effect on growth and on the alimentary tract. Johns Hopkins Hosp. Bul. 73:313.

HIGH LEVELS OF DRIED WHEY POWDER IN THE DIET OF SWINE

D. E. Becker, S. W. Terrill, A. H. Jensen and L. J. Hanson [1]
Illinois Agricultural Experiment Station

DRIED whey as an animal feed ingredient has been studied principally as a source of unidentified growth factors for poultry and swine. Recently Fisher *et al.* (1954) and Gard et al. (1955) have published papers pertaining to the value of dried whey as a source of unidentified factors for the chick and pig, respectively.

As dried whey normally contains about 70% lactose it seems logical that it should be used primarily as an energy source. However, with swine there has been a hesitancy to feed high levels of dried whey since severe diarrhea frequently ensues. For instance, Krider *et al.* (1949) reported the occurrence of diarrhea in weanling swine fed 4% to 8% of a dried whey product, and they felt that lactose was the causative factor.

Recently, Becker *et al.* (1954a, 1954b) reported a series of tests in which the nutritive value of various carbohydrates was determined with the pig at two different ages. In the baby pig, lactose fed at 56.6% of the diet produced a rate and efficiency of gain superior to either glucose or starch, without any evidence of diarrhea. Likewise, with the finishing pig, feeding 25% lactose promoted satisfactory rate and efficiency of gain with an absence of diarrhea. Hence, it seems that a minimum of 35% dried whey could be fed to finishing swine without the occurrence of a lactose-induced diarrhea.

The objective of the studies reported here was to study the carbohydrate replacement value of high levels of dried whey when fed to swine at various stages of growth.

Experimental

Four feeding trials were conducted with pigs produced in the University of Illinois herd. Prior to the start of the test all pigs were fed adequate diets. A description of the experimental diets is given in table 1.

Twenty-four baby pigs approximately 14 days of age, of Duroc and Yorkshire breeding, were used in Experiment 1. Eight outcome groups of three pigs were formed on the basis of ancestry, weight and general condition. The pigs of each outcome group were allotted at random to the whey levels. The pigs within each treatment were fed *ad libitum* in pairs in

[1] The authors wish to acknowledge Midwest Dried Milk Company, Dundee, Illinois; American Cyanamid Company, Pearl River, New York; Commercial Solvents Corporation, Terre Haute, Indiana; and Dow Chemical Company, Midland, Michigan, for funds and products which made this study possible.

metal, wire-bottom cages which were equipped with self feeders and waterers. The feeding period was 28 days.

In Experiment 2, thirty-six Duroc and crossbred pigs about 4 months of age were employed. Outcome groups of six pigs were formed on the basis of ancestry, weight and general condition. The pigs of each outcome group were randomly assigned to the whey treatments. In addition, pairs of outcome groups were divided randomly between the antibiotic treatments. Groups fed antibiotics received 25 mg. per pound of equal parts chlortetra-

TABLE 1. PERCENTAGE COMPOSITION OF DIETS FED

Experiment number	1	2	3	4
Ingredients:				
Soybean oil meal	—	30.0	—	30.8
Crude casein	25.0	—	17.0	—
Ground yellow corn	—	—	—	66.0
Dextrose	64.2	—	24.3	—
Dried whey [a]	—	61.3	30.0	[a]
Corn starch	—	—	20.0	—
Mineral mixture #2 [b]	4.0	4.0	4.0	—
Mineral mixture #3 [c]	—	—	—	2.0
Steamed bone meal	—	—	—	1.0
Woodflock	3.0	2.0	2.0	—
Corn oil	3.0	2.0	2.0	—
Vitamin A and D oil (3000A-600D)	0.5	0.5	0.5	—
Vitamin A and D concentrate (4000A-500D)	—	—	—	0.2
DL-methionine	0.3	0.2	0.2	—
Vitamin mixture #26 [d]	+	—	+	—
Vitamin mixture #37 [b]	—	+	—	+
Antibiotic [e]	+	—	—	+

[a] In Experiments 1 and 2, dried whey was combined with dextrose or corn starch, respectively, to yield the total quantities indicated. In Experiment 4, levels of dried whey replaced ground yellow corn.
[b] Becker and Terrill (1954).
[c] Becker *et al.* (1953).
[d] Becker *et al.* (1954c).
[e] Chlortetracycline HCl at 100 mg. per pound and procaine penicillin at 10 mg. per pound were fed in Experiments 1 and 4, respectively.

cycline HCl and procaine penicillin. The pigs were full-fed individually in feeding crates twice daily for 35 days.

Thirty-six pigs approximately 8 weeks of age, of Hampshire and Duroc breeding, were used in Experiment 3. Outcome groups of nine pigs were formed on the basis of breed, weight and general condition and the pigs within each group were distributed at random to the treatments. The pigs were full-fed individually twice daily for 42 days.

For the last test, thirty-two crossbred pigs about 8 weeks of age were used. The pigs were randomly assigned to the whey levels from outcome groups of four pigs each. They were group-fed *ad libitum* for a period of 34 days.

The various dried whey samples compared in Experiment 3 were pre-

pared in nine different plants. The dried whey employed in Experiments 1, 2 and 4 was a roller-dried product resulting from a cheddar cheese operation, and had the following average analysis: Crude protein, 13.0%; Crude fiber, 0.06%; Ether extract, 1.29%; Ash, 8.66%; Lactose (as lactose hydrate), 70.8%; Acidity (as lactic acid), 2.1%; Calcium, 0.75%; and Phosphorus, 0.62%.

Results

The results of Experiment 1 are presented in table 2.

With pigs weighing about 8 lb. initially no beneficial or deleterious effect of feeding dried whey at either 30% or 60% of the diet was observed.

TABLE 2. RESPONSE OF THE YOUNG PIG TO LEVELS OF DRIED WHEY IN A SEMI-PURIFIED DIET

(Experiment 1)

Experimental results			
Dried whey fed, %	0	30	60
No. of pigs	8	8	8
Av. initial wt., lb.	8.05	8.20	8.35
Av. daily gain, lb.	0.50	0.57	0.53
Feed per lb. gain, lb.	1.63	1.64	1.75

Analyses of variance

	Daily gain		Feed per lb. gain	
Source of variance	d/f	Mean square	d/f	Mean square
Levels of dried whey (L)	2	0.0150	2	0.0158
Outcome groups (G)	7	0.0207*	3	0.0260
L x G	14	0.0051	6	0.0060

* Significant at 5% level.

Diarrhea was not noted at any time during the trial. Although the pigs receiving dried whey gained slightly faster than the controls, this difference was not statistically significant. Likewise, there were no statistically significant differences in the amount of feed required per pound of gain. The analyses of variance for daily gain and feed efficiency show only that the outcome groups were significantly different ($P=0.05$) in daily gain.

The summary and the analyses of variance of daily gain, daily feed intake and feed efficiency for Experiment 2 are presented in tables 3 and 4, respectively.

There was no interaction of antibiotic and whey upon daily gain, daily feed intake or feed efficiency. The levels of dried whey produced an effect upon the rate of gain and the daily feed intake. Particularly, the rate of gain was markedly depressed by feeding 60% dried whey. There was

TABLE 3. RESPONSE OF THE FINISHING PIG TO LEVELS OF DRIED WHEY IN A SEMI-PURIFIED DIET

(Experiment 2)

Dried whey fed, %	0	5	10	20	40	60	Av.
Av. initial wt., lb.							
— Antibiotic	84.7	85.3	83.3	84.3	84.3	82.7	84.1
+ Antibiotic	86.3	83.0	81.3	83.7	85.0	81.3	83.4
Av.	85.5	84.2	82.3	84.0	84.7	82.0	83.8
Av. daily gain, lb.							
— Antibiotic	1.75	1.50	1.81	1.60[a]	1.67	1.02	1.56
+ Antibiotic	1.75	1.78	1.86	1.73	1.42	1.29	1.64
Av.	1.75	1.64	1.83	1.67	1.54	1.15	1.60
Av. daily feed intake, lb.							
— Antibiotic	4.30	4.04	4.48	4.36[a]	4.26	2.66	4.02
+ Antibiotic	4.21	4.47	4.23	4.47	3.89	2.87	4.02
Av.	4.26	4.25	4.35	4.41	4.08	2.76	4.02
Feed per lb. gain, lb.							
— Antibiotic	2.46	2.69	2.47	2.72[a]	2.56	2.61	2.59
+ Antibiotic	2.40	2.51	2.28	2.57	2.74	2.23	2.46
Av.	2.43	2.59	2.37	2.65	2.64	2.40	2.51

[a] Includes a calculated value for one pig that died during the trial.

some evidence that the daily gains had been depressed by 40% dried whey in the presence, but not in the absence, of antibiotics. Although 10% dried whey produced the most rapid gains, statistical analysis did not reveal that it was significantly different.

The values for daily feed consumption reveal that the highest level of dried whey produced a marked decrease in feed intake. Actually, the average intake of pigs fed 60% dried whey is only 69% of the average intake of all pigs on the test. Also, 40% of dried whey appeared to decrease feed intake in the presence of antibiotics, although there was no noticeable effect in the absence of antibiotics. Feed intake was greatest in those groups

TABLE 4. ANALYSES OF VARIANCE OF FEED AND GAIN DATA IN EXPERIMENT 2

Source of variance	d/f	Mean square		
		Daily gain	Daily feed intake	Feed per lb. gain
Antibiotic level (A)	1	0.0584	0.0002	0.1382
Pair groups (P)	2	0.0521	0.1492	0.0224
A x P	2	0.0269	0.1858	0.0305
Whey levels (W)	5	0.3450**	2.3504**	0.0948*
W x A	5	0.0570	0.1354	0.0519
W x P	10	0.0198	0.1310	0.0264
W x A x P	9	0.0278	0.0856	0.0231

* Significant at 5% level; ** Significant at 1% level.

TABLE 5. RESPONSE OF THE WEANLING PIG TO SAMPLES OF DRIED WHEY FED AS 30% OF A SEMI-PURIFIED DIET
(Experiment 3)

Sample description	No. of pigs	Av. initial wt.	Av. daily gain	Av. daily feed	Feed per lb. gain
		lb.	lb.	lb.	lb.
Spray-dried					
A	4	33.0	1.28	3.11	2.43
B	4	34.8	1.05	2.74	2.60
C	4	32.2	1.24	2.87	2.31
D	4	33 5	1.11	2.83	2.55
Av.		33.4	1.18	2.89	2.46
Roller-dried					
E	4	34.0	1.35	3.25	2.41
F	4	38.2	1.42	3.47	2.44
G	4	33.5	1.31	3.18	2.43
H	4[a]	37.0	1.37	3.28	2.40
I	4	31.0	1.33	3.04	2.29
Av.		34.8	1.36	3.24	2.39

[a] Includes a calculated value for a pig removed from the test.

fed 10% or 20% dried whey. Figures for efficiency of feed conversion do not follow any clear pattern. The least efficient gains were noted at the 20% and 40% levels. Feed efficiency at 60% dried whey was practically the same as that at 10% dried whey, which was the most efficient group. The analyses of variance show that the whey levels exerted a statistically significant effect on daily gains (P=0.01), daily feed intake (P=0.01) and feed per pound of gain (P=0.05).

It was observed that feeding 60% dried whey caused a marked diarrhea, and there was some evidence of diarrhea at the 40% level. Lower levels of dried whey did not induce diarrhea, except for some mild cases about 2 days after the start of the test.

The summary and the analyses of variance of daily gain, daily feed

TABLE 6. ANALYSES OF VARIANCE OF FEED AND GAIN DATA IN EXPERIMENT 3

Source of variance	d/f	Mean square		
		Daily gain	Daily feed intake	Feed per lb. gain
Drying method	1	0.2816*	1.1052**	0.0845
Within roller-dried	4	0.0077	0.1021	0.0118
Within spray-dried	3	0.0388	0.0930	0.0864
Outcome groups	3	0.0273	0.5076**	0.0944*
Groups x treatments	23	0.0562	0.1003	0.0305

* Significant at 5% level; ** Significant at 1% level.

intake and feed efficiency for Experiment 3 are presented in tables 5 and 6, respectively.

Performance of the pigs fed the various samples of dried whey was satisfactory in all cases. However, pigs fed the roller-dried samples made 13% greater gains and consumed 12% more feed than the pigs fed the spray-dried wheys. There was practically no difference in the amount of feed required per pound of gain. Hence, it appears that most of the difference in response to processing technique resulted from differences in total feed consumed. A greater variation in rate and efficiency of gains occurred within the spray-dried samples than within the roller-dried, although the wheys within each drying method did not differ significantly. It is notable that the best daily gain obtained on a spray-dried whey failed

TABLE 7. RESPONSE OF THE WEANLING PIG TO LEVELS OF DRIED WHEY IN A PRACTICAL DIET

(Experiment 4)

Experimental results				
Dried whey fed, %	0	10	20	30
No. of pigs	8	8	8	8
Av. initial wt., lb.	41.2	41.4	39.2	38.2
Av. daily gain, lb.	1.38	1.34	1.12	1.14
Feed per lb. gain, lb.	2.66	2.39	2.73	2.49

Analysis of variance

	Average daily gain	
Source of variance	d/f	Mean square
Levels of dried whey (L)	3	0.1346**
Outcome groups (G)	7	0.0622**
L x G	21	0.0150

** Significant at 1% level.

to equal the poorest performance on the roller-dried wheys. The analyses of variance reveal that the drying method produced a statistically significant effect on daily gains (P=0.05) and daily feed intake (P=0.01).

An accurate record was kept on occurrence of diarrhea during the trial. A flurry of diarrhea occurred in all pigs, regardless of treatment, during the initial 2 or 3 days of the test. Afterwards, diarrhea was limited to an occasional soft stool which had no particular association with the treatments. There was some evidence that the pigs on the highest feed intake were the ones most inclined to excrete soft stools.

The results of Experiment 4 are presented in table 7. Incorporation of 10% dried whey as a replacement for yellow corn had practically no effect upon the daily gains; however, both 20% and 30% dried whey caused a marked depression in daily gain. The depression produced by the two

highest whey levels was of about the same magnitude. Values for feed required per pound of gain do not conform to any recognizable pattern. The significance of the differences cannot be tested statistically, but it appears that the levels of dried whey, even the 30% level, did not markedly depress the efficiency of feed conversion. Severe diarrhea was not encountered, although all treatments caused the pigs to excrete soft feces. Analysis of variance shows that the levels of dried whey exhibited a significant effect ($P=0.01$) upon the daily gains.

Discussion

The studies reported here indicate that the pig has the ability to utilize higher levels of dried whey, without deleterious effect, than some workers have reported. However, the maximum advisable feeding rate appears to depend primarily on the remainder of the diet and the age of the pig. In a semi-purified diet using casein plus methionine as the source of protein, the suckling-age pig can utilize 60% dried whey in the diet. A 40% level is about the maximum for the finishing pig fed a semi-purified diet with soybean oil meal plus methionine as the source of protein. On the other hand, the weanling pig can not tolerate much more than 10% of dried whey in a practical diet. The latter observation is more in agreement with the finding of Krider *et al.* (1949), who reported that 4% or 8% of a dried whey product produced some depression of gain and feed intake in weanling pigs fed a diet composed largely of corn and soybean oil meal. In view of our results, it is interesting that Bünger *et al.* (1939) reported that dried whey could be successfully fed to swine as a supplement to a regular diet of potatoes, grain mixture and fishmeal provided the daily intake of whey did not exceed about 1.1 lb. Larger amounts tended to depress the appetite of the animals. In the studies presented here the baby pig and the finishing pig utilized 1.0 and 1.6 lb., respectively, of dried whey daily, but the weanling pig fed a practical diet could utilize only 0.32 lb. of dried whey daily.

It is noteworthy that the more mature pig has the ability to utilize efficiently significant quantities of dried whey in the diet, since Bailey *et al.* (1955) reported that the lactase activity of the intestinal mucosa of swine decreases sharply by seven weeks of age.

It is clear that the differences in response to high levels of dried whey cannot be explained solely in terms of the level of lactose fed. Becker *et al.* (1954a, 1954b) found that the baby pig and the finishing pig can tolerate at least 57% and 25% respectively, of lactose in the diet without noticeably affecting the rate and efficiency of gain or the incidence of diarrhea. Hence, the baby pig could theoretically tolerate the lactose contributed by 80% of dried whey in the diet; whereas, 25% lactose for the finishing pig would indicate a maximum of 36% dried whey in the diet. Of course, the lactose maxima suggested here are far greater than the levels supplied by

Krider *et al.* (1949) in a practical diet containing relatively low levels of dried whey. Although it is true that lactose and dried-whey tolerance both show an indirect relationship with the age of the pig, the tolerances are probably established by different mechanisms.

A possibility for the mechanism involved in fixing dried whey tolerance may be indicated in the study of Daniel and Harvey (1947). They reported that dialysis of whey produced a beneficial effect on its nutritive value for rats. In addition, whey ash depressed the growth of rats fed dried whole milk and dialyzed whey as the source of protein. These observations implicate the inorganic fraction of dried whey as the growth-retarding factor. Hence, the response from feeding high levels of dried whey might well depend upon the mineral composition of the remainder of the diet. Certainly the semi-purified diets used here have a mineral composition different from that of practical diets in which yellow corn and soybean oil meal are the principal ingredients. Further studies are underway to explore the importance of inorganic components in diets bearing high levels of dried whey.

The merit of the roller-dried samples compared to the spray-dried products was quite unexpected since the latter process is generally believed to yield a superior product. For instance, Riggs *et al.* (1955) reported that the protein in spray-dried whey powder was more digestible and had a higher biological value than that in roller-dried whey powder. In our studies the superiority of the roller process appeared to be a consequence of increased feed consumption. Failure to find a significant difference in feed conversion may indicate a similar nutritional value of the dried whey powders prepared by the two processes when the whey is used as a carbohydrate replacement.

Summary

High levels of dried whey as a carbohydrate replacement were studied in swine at various stages of growth.

With the suckling pig (8 lb.) fed a synthetic diet, with casein plus methionine as the source of protein, levels of 0, 30 and 60% dried whey were studied as a substitute for dextrose. There was no significant difference in rate and efficiency of gain, nor any evidence of a whey-induced diarrhea.

With the finishing pig (100 lb.) levels of 0, 5, 10, 20, 40 and 60% dried whey were studied as a starch replacement both in the presence and absence of an antibiotic. A semi-synthetic diet with soybean oil meal plus methionine as the source of protein was used. Level of dried whey did not interact with antibiotic. A level of 60% dried whey produced a depression in rate of gain and daily feed intake and a marked diarrhea, but there was no effect upon the efficiency of gain. All other levels of whey yielded satisfactory performance, although there was some evidence of diarrhea at the 40% level.

With weanling pigs, incorporation of 20 or 30% dried whey into the diet as a substitute for corn in a corn-soybean oil meal diet produced a marked decrease in rate of gain and feed intake and a moderate diarrhea.

Samples of roller-dried whey produced a higher rate of gain and feed intake than samples of spray-dried whey when fed to the weanling pig as 30% of a diet which contained casein plus methionine as the source of protein. Drying method did not affect feed efficiency.

Literature Cited

Bailey, C. B., W. D. Kitts and A. J. Wood. 1956. The development of the digestive enzyme system of the pig during its pre-weaning phase of growth. B. Intestinal lactase, sucrase, and maltase. Canadian J. Agr. Sci. 36:51.

Becker, D. E., S. W. Terrill, J. W. Lassiter, T. S. Nelson and D. I. Gard. 1953. The influence of antibiotic combinations on the growth response of the pig. Antibiotics and Chemotherapy 3:778.

Becker, D. E., D. E. Ullrey and S. W. Terrill. 1954a. A comparison of carbohydrates in a synthetic milk diet for the baby pig. Arch. Biochem. Biophys. 48:178.

Becker, D. E. and S. W. Terrill. 1954b. Various carbohydrates in a semi-purified diet for the growing pig. Arch. Biochem. Biophys. 50:399.

Becker, D. E., D. E. Ullrey and S. W. Terrill. 1954c. Protein and amino acid intakes for optimum growth rate in the young pig. J. Animal Sci. 13:346.

Bünger, H., E. Fissmer, W. Harre, H. Schmidt, K. Boehm and F. Reising. 1939. Feeding trials with dried whey. Biedermans. Tentbl. Abt. B., Tierernahrung 11:276.

Daniel, F. K. and E. H. Harvey. 1947. Some observations on the nutritional value of dialyzed whey solids. J. Nutr. 33:429.

Fisher, H., H. M. Scott and R. G. Hansen. 1954. Further studies on the alfalfa factor and its relation to the liver and whey factors. J. Nutrition 52:13.

Gard, D. I., D. E. Becker, S. W. Terrill, H. W. Norton and A. V. Nalbandov. 1955. Sources of unidentified factors for the pig. J. Animal Sci. 14:532.

Krider, J. L., D. E. Becker, L. V. Curtin and R. F. VanPoucke. 1949. Dried whey products in drylot rations for weanling pigs. J. Animal Sci. 8:112.

Riggs, L. K., A. Beaty and B. Mallom. 1955. Nutritive value of whey powder protein. J. Agr. Food Chem. 3:333.

THE VALUE OF FORTIFIED CREEP RATIONS FOR SINGLE AND TWIN SUCKLING LAMBS[1]

T. W. Perry, W. M. Beeson and Claude Harper

Purdue University, Agricultural Experiment Station, Lafayette, Indiana

RATE of gain and economy of gain of lambs are optimum at the time when the lambs are still quite young and are nursing their mothers. As lambs approach weaning age and weight, their rate of growth decreases and eventually such factors as poor nutrition, summer heat, parasities and drouthy pastures may slow down the growth rate even more.

Since lambs make quite rapid gains early in life, it was desirable to investigate the possibility of further increasing their growth rate early in life so the majority of them would reach market weight at a young age.

Research conducted more than a half century ago (Wisconsin, 1895, 1897, 1898, 1899) was designed to determine the value of creep rations for suckling lambs in drylot and on pasture. This early work consisted mainly of comparing either corn or oats, alone, with a mixture of the two. In subsequent research, the comparative value of creep feeding suckling lambs corn alone or a combination of corn, oats and linseed oil meal showed corn alone to be as valuable as the mixture (Garrigus, 1951). In more recent research, Purdue University reported no economic benefit from feeding creep rations to lambs on pasture, in one trial, but in a later trial reported growth stimulation from creep-feeding on pasture (Harper, 1930, 1933; Indiana Agr. Expt. Station Annual Report, 1936).

In the past two decades research has revealed the role of many factors in the early life of the pig, the chicken and the calf, such as antibiotics, B-vitamins and hormones. Since the addition of many of these factors has resulted in growth stimulation and improved utilization of feed, it was desirable to determine the effect of more highly fortified creep rations for the suckling lamb.

Experimental

Trial I. Starting with the birth of the first lamb from Hampshire x Northwest crossbred ewes, about February 1, single lambs were assigned in order of birth, without respect to sex or birthweight, to one of six lots. In the first trial, twins weighing within one pound of the other at birth, were assigned to one of two lots. When the two twin lots had been filled (46 and 44 lambs, respectively) the smaller in each subsequent pair was destroyed and the larger lamb was treated as a single.

All lambs had access to their respective creep rations in a separate pen or "creep" in which water was available, and over which an infra-red

[1] Contribution from the Department of Animal Husbandry, Journal Paper No. 1040.

heat lamp was suspended so that the creep was comfortable and attractive to the lambs. The creep ration was available from the third day of the lamb's life—or at the time the lambs and mother were transferred from the maternity pens to their respective lots.

The lambs were weighed individually at 14-day intervals. On April 20, 1955, they were turned to legume pasture with the ewes with no creep ration available. The composition of the rations is listed in table 1. The

TABLE 1. DEHYDRATED ALFALFA MEAL IN THE PELLETED CREEP RATION OF SUCKLING LAMBS (1955)

	Singles						Twins	
	Lot I	Lot II	Lot III	Lot IV	Lot V	Lot VI	Lot VII	Lot VIII
Percentage composition of rations								
Dehydrated alfalfa meal	0	20	40	60	80	98	0	40
Cane sugar	0	0	0	0	0	0	10	10
Gr. yellow corn, %	88	68	48	28	8	0	77.5	37.5
Soybean oil meal, %	10	10	10	10	10	0	10	10
Bone meal, %	1	1	1	1	1	1	1	1
Iodized salt, %	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Premix, %	0.5[a]	0.5[a]	0.5[a]	0.5[a]	0.5[a]	0.5	1[b]	1[b]
Drylot phase, creep rations available (Feb. 1 to April 20, 1954)								
Number of lambs	17	19	16	18	14	18	46	44
Birth wt., lb.	10.7	12.0	11.3	10.6	11.4	12.0	9.9	10.1
Final wt., lb.	60	61	60	53	58	57	54	52
Av. daily gain, lb.[c]	0.78	0.76	0.76	0.68	0.71	0.75	0.63	0.61
Creep feed consumption, lb. per day	0.52	0.45	0.53	0.49	0.58	0.49	0.62	0.66
Creep feed per 100 lb. gain, lb.	66	59	71	72	80	66	98	110
Subsequent pasture phase, running with mother, no creep ration available (April 20–July 1, 1954)								
Av. daily gain, lb. (pasture)	0.57	0.52	0.54	0.53	0.58	0.55	0.53	0.51
Av. daily gain, lb. (both phases)	0.69	0.64	0.64	0.61	0.66	0.65	0.58	0.56

[a] Premix contributed 10 gm. mixed tocopherols, 5 million units Vitamin A, and 625,000 units of Vitamin D per ton of ration.

[b] Premix contributed the following to each ton of feed: chlortetracycline, 40 gm.; Thiamine, 2 gm.; Riboflavin, 4.0 gm.; Niacin, 32 gm.; Calcium pantothenate, 20 gm.; Vitamin B_{12}, 40 mg.; Ascorbic Acid, 100 mg.; Mixed tocopherols, 10 gm.; Vitamin A, 5 million units; Vitamin D, 625 thousand units; $FeSO_4 \cdot 7H_2O$, 150 gm.; $CuSO_4 \cdot 5H_2O$, 12 gm.; and $MnSO_4 \cdot 7H_2O$, 90 gm.

[c] Single lambs gained significantly more than twins.

six lots of single lambs had access to pelleted creep rations containing various levels of dehydrated alfalfa meal (17% crude protein), which was the variable constituent. The levels of dehydrated alfalfa meal in the creep rations of the six lots were respectively, 0, 20%, 40%, 60%, 80%, and 98%, 1% bone meal; 0.5% iodized salt; 0.5% of a premix containing Vitamins A, D, and E, and ground corn and soybean oil meal constituted the remainder of the ration.

Lots VII and VIII contained only the twin lambs born in the flock. Pellets fortified with cane sugar, chlortetracycline, thiamine, riboflavin, niacin, calcium pantothenate, vitamin B_{12}, ascorbic acid, vitamins A, D, and E, and iron, copper and manganese were fed in the twin lots.

TABLE 2. THE VALUE OF A HIGHLY FORTIFIED CREEP RATION FOR SINGLE AND TWIN SUCKLING LAMBS (1956)

	Twins		Singles			
	Lot I 40% alfalfa	Lot II "Super"	Lot III No alfalfa	Lot IV 40% alfalfa	Lot V 98% alfalfa	Lot VI "Super"
Percentage composition of pelleted creep ration						
Ground yellow corn	48	37.5	88	48	0	37.5
Soybean oil meal	10	10	10	10	0	10
Dehydrated alfalfa meal	40	40	0	40	98	40
Cane sugar	0	10	0	0	0	10
Bone meal	1	1	1	1	1	1
Iodized salt	0.5	0.5	0.5	0.5	0.5	0.5
Premix	0.5[a]	1[b]	0.5[a]	0.5[a]	0.5[a]	1[b]
Drylot phase creep ration available (Feb. 1 to May 4, 1956)						
Number of lambs	48	52	20	20	19	20
Birth weight, lb.	10.0	9.7	11.2	11.8	12.2	11.6
Wt. May 4, lb.	59	67	78	72	71	77
Daily gain, lb.[c]	0.63	0.75	0.84	0.77	0.73	0.83
Creep feed consumption per day, lb.	0.90	1.15	1.00	0.97	1.03	1.12
Creep feed per 100 lb. gain, lb.	142	152	119	126	142	135
Lamb quality, May 4,[d]						
Blue lambs, %	0	94	100	95	42	95
Red lambs, %	41	4	0	5	37	5
Yellow lambs, %	42	2	0	0	5	0
Green lambs, %	17	0	0	0	0	0
Culls, %	0	0	0	0	16	0
Subsequent pasture phase, running with mother, Lot VI ration available to all lambs (May 4 to June 15)						
Weight, June 15, lb.	86	92	103	101	96	102
Daily gain lb. (Pasture phase)	0.65	0.59	0.59	0.68	0.59	0.60
Daily gain lb. (Both phases)	0.64	0.70	0.75	0.74	0.68	0.75
Lamb quality, June 15, (Selling price)						
Blue lambs, % ($23.00 per cwt.)	32	65	90	95	74	84
Red lambs, % ($22.00 per cwt.)	49	29	10		10	11
Yellow lambs, % ($20.50 per cwt.)	15	6	—	5	16	5
Green lambs, %	4	—	—	—	—	—

[a] Premix contributed 10 gm. mixed tocopherols; 5 million units vitamin A; and 625,000 units of vitamin D per ton of ration.

[b] Premix contributed the following to each ton of feed; chlortetracycline, 40 gm.; thiamine, 2 gm.; riboflavin, 4.0 gm.; niacin, 32 gm.; calcium pantothenate, 20 gm.; vitamin B_{12}, 40 mg.; ascorbic acid, 100 gm.; mixed tocopherols, 10 gm.; vitamin A, 5 million units; vitamin D, 625,000 units; $FeSO_4 \cdot 7H_2O$, 150 gm.; $CuSO_4 \cdot 5H_2O$, 12 gm.; and $Mn\ SO_4 \cdot 7H_2O$, 90 mg.

[c] At the 1% level of statistical significance all other lots gained more than I, III and VI gained more than II or V.

[d] Blue lambs—strictly choice; Red lambs—good to low choice; Yellow lambs—medium; Green lambs—below medium or feeder grade.

Trial II. In Experiment II the single and twin lambs were assigned to their respective lots on the basis of date of birth as indicated in Trial I. The composition of the various creep pellets is indicated in table 2. The pellets available in three of the four lots of single lambs varied in the level of dehydrated alfalfa meal contained—0, 40%, or 98%. A fourth lot

of single lambs had access to "super" corn-soy-alfalfa pellet fortified with sugar, antibiotics, vitamins and trace minerals, (Lot VI).

The creep rations for the two lots of twin lambs differed in that one contained the relatively simple formula of feedstuffs, whereas the other contained the "super" fortified creep diet, (Lot II).

All lambs and ewes were turned out to legume pasture together on May 4, 1956. The lambs were separated from their mothers for a period of six or seven hours each day and had access to the "super" fortified pellet (used in Lots II and VI during the drylot phase) during this time.

The lambs were graded individually, consigned to a lamb pool and graded at the conclusion of the trial (June 15).

The data in both trials were studied using analysis of variance.

Results

Trial I. Pellets composed of 98% dehydrated alfalfa meal (Lots VI, table 1) were just as valuable as a creep ration for suckling lambs as pellets containing 10% soybean oil meal and varying amounts of corn (Lots I through V). There was very little difference in consumption between the six lots of single lambs, indicating that higher levels of dehydrated alfalfa meal did not affect palatability adversely. Apparently the dehydrated alfalfa meal had a 100% corn-replacement value in these pellets, in that lambs grew as rapidly and efficiently on pellets containing higher levels of dehydrated alfalfa meal as those on pellets containing much lower levels.

Lots VII and VIII contained only the twin lambs born in the flock. Highly fortified pellets, differing only in level of corn replaced with dehydrated alfalfa meal, (40%) were available in the two twin lots. Since the two lots grew at approximately the same rate, dehydrated alfalfa meal apparently had a 100% corn-replacement value, at a level of 40% of the ration, for twin lambs. Single lambs gained significantly more than twin lambs.

Type of creep pellets had no significant effect on the gain of lambs in the subsequent pasture phase, when the lambs were nursing their mothers with no creep ration available. As was the case in drylot, twins did not gain as rapidly during the pasture phase as single lambs.

Trial II. Single lambs receiving creep pellets containing corn, soybean oil meal, salt, bone meal and vitamins A, D, and E (Lot III, table 2) grew as rapidly as lambs fed similar pellets (Lot VI) fortified with 10% sugar, an antibiotic, B-vitamins, ascorbic acid and trace minerals. Both lots gained significantly more ($P<0.01$) than lambs receiving pellets containing 40% (Lot IV) or 98% (Lot V) dehydrated alfalfa meal. This is contrary to the results obtained in the previous trial in which the higher levels of dehydrated alfalfa meal replaced corn pound for pound.

Twin lambs which had access to "super" pellets (Lot II) grew as rapidly as single lambs fed pellets containing either 40%, or 98% alfalfa (0.75 lb.

vs. 0.77 and 0.73 lb. per day) but not as rapidly as single lambs receiving pellets containing no dehydrated alfalfa meal (Lot III, 0.84 lb.) or those receiving "super" pellets (Lot VI, 0.83 lb.).

Single lambs having access to pellets containing 0, 40, or 98% dehydrated alfalfa meal consumed about the same amount per day (0.97 to 1.03 lb. per lamb) but less than the single lambs which had access to the "super" pellet (1.12 lb. per lamb).

At the time the lambs were turned to pasture with their mothers (May 4), the twin lambs which had access to fortified pellets (Lot II) graded higher than the twins which had received unfortified pellets (Lot I). In the subsequent pasture period, in which all lambs had access to the fortified pellet, the difference between Lot 1 and Lot II became smaller, although Lot 1 completed the experiment (June 15) with lower carcass grade and lower overall average daily gain.

A markedly higher live grade was evident for the twin lambs which received super pellets (Lot II) over those which received regular 40% dehydrated alfalfa meal pellets (Lot I). This difference was greatest at the end of the drylot period (May 4), and the difference decreased during the pasture period, in which all lots of lambs had access to pasture with their mother approximately 18 hours per day and had access to "super" pellets the other 6 hours per day.

In the single lambs, the most marked effect on live grade was that the lambs which received 98% alfalfa meal pellets (Lot V) did not have the finish of the other three lots of lambs at the end of the drylot period.

Summary

Two trials are summarized in which suckling lambs in drylot with their mothers had access to creep pellets of differing composition.

In the first trial, dehydrated alfalfa meal was incorporated in the creep pellets for single lambs at levels of 0, 20%, 40%, 60%, 80%, or 98%. Level of alfalfa had no effect on growth rate, nor on consumption of creep pellets. Also, two lots of twin lambs had access to "super" pellets fortified with sugar, B-vitamins, vitamins A, D, E, and C, an antibiotic, iron, copper and manganese. The pellets for one lot had 40% of the corn replaced with dehydrated alfalfa meal whereas pellets for the second lot contained no dehydrated alfalfa meal. Level of alfalfa meal had no significant effect on growth rate or pellet consumption.

In the second trial, single lambs had access to creep pellets containing 0, 40%, or 98% dehydrated alfalfa meal, or "super" pellets containing 40% dehydrated alfalfa meal. Single lambs which consumed pellets containing no dehydrated alfalfa meal, or those containing the "super" ration gained at the same rate (0.84 lb. vs. 0.83 lb. per day), and significantly more than those consuming pellets containing either 40% (0.77 lb. per day) or 98% dehydrated alfalfa (0.73 lb. per day).

In the second trial twin lambs fed "super" pellets gained more than those fed pellets not containing the extra ingredients of the "super" pellets.

Literature Cited

Garrigus, W. P. 1951. Concentrates for creep-fed lambs. Kentucky Agr. Exp. Sta. Bul. 567.

Harper, Claude. 1930. Producing native lambs. Indiana Agr. Exp. Sta. Bul. 344.

Harper, Claude. 1933. The value of feeding grain to unweaned lambs on pasture. American Society of Animal Production Proc. P. 157.

Indiana Agr. Exp. Sta. Annual Report. 1936.

Missouri Agr. Exp. Sta. Annual Report. 1934. P. 13; 1935, P. 11.

New York Agr. Exp. Sta. Annual Report. 1935. P. 80.

Oklahoma Agr. Exp. Sta. Annual Report 1938–40. P. 59.

Perry, T. W., W. M. Beeson and Claude Harper. 1955. Dehydrated alfalfa meal in creep rations of suckling lambs. Purdue Agr. Exp. Sta. Mimeo A. H. 159.

Perry, T. W., W. M. Beeson, W. H. Smith and Claude Harper. 1956. The value of highly fortified creep rations for single and twin lambs. Purdue Agr. Exp. Sta. Mimeo A. H. 177.

Weber, A. D. 1930. Raising early lambs from aged western ewes. Nebraska Agr. Exp. Sta. Bul. 250.

Wisconsin Agr. Exp. Sta. Annual Report 1895, P. 40; 1897, P. 37 and 42; 1898, P. 17; 1899, P. 44.

THE UTILIZATION OF AMMONIATED CANE MOLASSES BY SHEEP

ALLEN D. TILLMAN, WILLIS D. GALLUP AND WALTER WOODS
Oklahoma Agricultural Experiment Station [1]

THE availability of nitrogen in ammoniated cane molasses (Stiles, 1952) is a consideration of first importance in the evaluation of this product as a feed for cattle and sheep. The question of possible toxicity is also involved.

In digestion and nitrogen balance studies with sheep, Tillman and Swift (1953) found ammoniated cane molasses (ACM) to be inferior to both urea and soybean oil meal when these products supplied 17% of the total ration nitrogen. In similar studies with dairy cattle, Davis *et al.* (1955) found that rations containing ammoniated feeds were inferior to soybean oil meal rations in nitrogen digestibility. Recently Tillman *et al.* (1957) determined with steers the digestibility of nitrogen in rations of prairie hay and ACM; digestibility of the ACM nitrogen was calculated by difference and found to be about 59%.

Several workers (Barrentine and Darnell, 1954; Richardson et al., 1954; Tillman *et al.*, 1957) have reported stimulation in cattle fed ACM. In some instances (Tillman *et al.*, 1957) the cattle reacted with violence and injured themselves by running into and attempting to climb fences in a manner of extreme excitement. The purpose of the present study was (1) to determine the digestibility of ACM rations fed to sheep, (2) to calculate by difference the digestibility of nutrients in ACM and (3) to observe the behavior of sheep fed high levels of this product.

Experimental Procedure

Trial 1, Metabolism. The daily basal ration, in grams, was composed of cottonseed hulls, 500; cane molasses, 166; sodium chloride, 4.5; dicalcium phosphate, 4.0; monosodium phosphate, 4.0 and vitamins A and D[2], 0.5. The supplemented ration was the same plus 66 grams of ACM (33% protein equivalent). The ACM supplement increased the protein content of the ration from 3.9% to 7.0%, dry matter basis (table 1).

Twelve western-type wether lambs weighing 70±3 lb. were drenched with a preparation containing phenothiazine 2 weeks prior to the trial. They were then divided into two groups, one group of six received the basal while the others received the supplemented ration. The sheep were kept in metabolism stalls (Briggs and Gallup, 1949) and fed one-half of their rations twice daily during successive 10-day preliminary and collec-

[1] Departments of Animal Husbandry and Agricultural Chemistry, Stillwater.
[2] Quadrex type IV. Supplied gratis by NOPCO Chemical Co., Harrison, N. J.

tion periods. Feces and urine were handled as described by Tillman and Swift (1953). Digestion coefficients were calculated by difference by the method of Forbes *et al.* (1943).

Trial 2, Metabolism. In this trial the basal ration contained 7.0% protein which was increased to 10.1% by the addition of ACM. The composition and daily intake of the basal ration, in grams, was cottonseed hulls, 450; cane molasses, 100; soybean meal, 44; sodium chloride, 4.2; dicalcium phosphate, 6.3; monosodium phosphate, 1.0 and vitamins A and D, 0.5. The supplemented ration contained, in addition, 66 grams of ACM (33%).

As in trial 1, 12 western-type wether lambs weighing 70±4 lb. were divided into two groups, one group of six being fed the basal ration and the others fed the supplemented ration. Other details were as described in trial 1.

TABLE 1. COMPOSITION OF RATIONS USED IN METABOLISM TRIALS AND OF SELECTED FEEDS

Rations and Feeds	Trial	Dry matter %	Percentage composition of dry matter				
			Ash	Protein (N x 6.25)	Ether extract	Crude fiber	N-free extract [a]
Basal ration	1	86.9	6.3	3.9	0.6	34.0	55.2
Basal ration plus ACM [b]	1	85.3	6.6	7.0	0.6	31.6	54.2
Basal ration	2	88.2	6.0	7.0	0.5	33.0	53.5
Basal ration plus ACM [b]	2	86.3	6.3	10.1	0.5	30.5	52.6
ACM (33%)	1,2,3	68.8	9.9	46.8	—	—	43.3
ACM (16%)	3	74.8	8.2	21.2	—	—	70.6
ACM (high test)	4	69.2	2.7	44.8	—	—	52.5
Cane molasses	1,2	73.1	10.1	5.3	—	—	84.6
Cane molasses (high test)	4	74.0	2.7	4.1	—	—	93.2

[a] Determined by conventional feed analysis.
[b] Ammoniated cane molasses, 33% protein equivalent.

Trial 3, Stimulation test. Ten western-type wether lambs weighing 60±2 lb. were divided into two groups of five each and fed high levels of two ammoniated cane molasses products, one containing 16% protein equivalent and the other 33%. The ration for one group contained, in percent, cottonseed hulls, 40.0; ACM (16%), 57.4; steamed bone meal, 2.0; vitamins A and D, 0.1 and salt, 0.5. The ration for the other group was the same except that ACM (33%) replaced the ACM (16%). This replacement increased the protein content of the ration, as fed, from about 10% to 19%. All ingredients were weighed at each feeding and the ACM poured over and mixed with the dry feed. The lambs were individually-penned and -fed the rations *ad lib.* for 7 days. They were observed periodically during the day for abnormal behavior.

At the end of 7 days the lambs were fasted for 48 hours and then given their respective rations for another 5-day period. For 24 hours after the fast was broken, the animals were under constant observation; thereafter they were observed periodically during the day.

Another group of three western-type wether lambs served as controls. They weighed 65±5 lb. and were fed cane molasses in place of the ACM.

Trial 4, High-test ACM in a fattening ration. Nine western-type wether lambs weighing 75±4 lb. were divided into three equal groups. One group was fed a low-protein basal ration; another group, the basal ration plus 147 gm. of ammoniated high-test cane molasses; and the third group, the basal ration plus 98 gm. of soybean oil meal. The daily basal ration, in gm., was composed of cottonseed hulls, 450; yellow corn meal, 387, high-test cane molasses, 100; salt, 5.0; calcium carbonate, 3.8; dicalcium phosphate, 3.5 and vitamins A and D, 0.7. It contained 5.8% protein (dry matter basis) which was increased to 10.1% in the supplemented rations. The high-test molasses was a Cuban product in which about 50% of the sucrose had been hydrolyzed to simple sugars. It contained more nitrogen-free extract and less nitrogen and ash than the regular blackstrap molasses.

The lambs were individually-fed the rations *ad lib.* for a 42-day period. They were weighed at 14-day intervals, the initial and final weights being taken after 16-hr. shrink periods. At the end of the trial the lambs were slaughtered and a gross examination made for pathological condition.

Results and Discussion

Trials 1 and 2. The results of the two digestion and nitrogen balance trial are shown in table 2. Data from three lambs that refused feed on each ration in trial 1 have not been included.

In trial 1, the addition of ACM (33%) to the low-protein basal ration (3.9%) resulted in a marked increase in all digestion coefficients. The ACM supplement, which supplied 48% of the total nitrogen, increased the digestibility of the major constituents in the basal ration to such an extent that despite increased intake, fecal excretion of dry matter was less on the supplemented ration than on the basal ration. Average daily dry matter intakes on the basal and supplemented rations were 591 and 636 gm., respectively; dry matter excretion was 369 and 316 gm., respectively. As a result, calculation by difference of the digestibility of dry matter, organic matter and nitrogen-free extract (conventional analysis) in the ACM yielded spurious values of over 200%. Since the ACM did not contribute any crude fiber or ether extract to the supplemented ration, increased values for the digestibility of these constituents, 39.3% and 60.9%, respectively, as compared to 24.7% and 36.2% on the basal ration, represent actual increases in the digestibility of these basal ration constituents.

The effect of ACM upon the digestibility of protein in the basal ration cannot be stated with certainty. The supplement provided, through an additional breakdown of crude fiber, opportunity for a more complete digestion of protein as well as a possible reduction in endogenous fecal protein. If, through this breakdown, the apparent digestibility of the basal protein was increased, the increase would be credited to the ACM in the

calculation of its digestibility by difference (Forbes *et al.*, 1943). Accordingly, the value of 49.9% for the apparent digestibility of protein in the ACM in trial 1 may be slightly higher than the actual value.

In trial 2, the same amount of ACM (33%) as fed in trial 1, when added to a basal ration of higher protein content (7.0%) resulted in a less marked increase in digestion coefficients; ether extract digestibility was decreased. In this trial, the digestibility of nutrients in the basal ration was high as compared to trial 1, the digestibility of dry matter and crude fiber

TABLE 2. DIGESTIBILITY AND NITROGEN BALANCE DATA [a]

Ration and product	Basal	Basal + ACM	ACM [b]	Basal	Basal + ACM	ACM [b]
Dry matter intake, gm.	590.7	636.1	45.4	534.5	579.9	45.4
Protein (N x 6.25), %	3.9	7.0	46.8	7.0	10.1	46.8
Trial No.	1	1	1	2	2	2
Digestibility, %						
Dry matter	37.6 (1.3)	50.3 (7.0)	—[c]	50.8 (5.1)	56.9 (2.4)	—[d]
Organic matter	36.3 (1.5)	49.3 (7.5)	—[c]	50.5 (5.1)	56.5 (2.3)	—[d]
Protein (N x 6.25)	—27.1 (4.0)	10.1 (4.0)	49.9 (8.2)	25.5 (9.0)	31.2 (2.4)	41.6 (7.2)
Ether extract	36.2 (10.8)	60.9 (4.0)	—	66.3 (9.6)	57.5 (7.7)	—
Crude fiber	24.7 (2.2)	39.3 (11.0)	—	40.8 (5.0)	47.6 (3.8)	—
N-free extract	47.6 (2.6)	56.5 (7.1)	—[c]	59.5 (6.0)	66.6 (1.2)	—[d]
Nitrogen, daily, gm.						
Intake	3.67	7.08	3.41	5.99	9.40	3.41
Feces	4.66	6.36	1.70	4.46	6.46	2.00
Urine	1.33	1.84	0.52	1.19	2.10	0.91
Balance	—2.32	—1.12	—[e]	0.34	0.84	—[f]
Retained, %	0.0	0.0	—	5.7	8.8	—

[a] Numbers in parentheses are standard deviations.
[b] Values for ACM calculated by difference.
[c] Values of over 200%.
[d] Values of over 100%.
[e] Difference in balance equivalent to 35.2% of ACM-nitrogen intake.
[f] Difference in balance equivalent to 14.7% of ACM-nitrogen intake.

being 50.8 and 40.8%, respectively. These higher digestibilities are ascribed to a more active rumen microflora (Williams *et al.*, 1953) resulting from the higher protein content of the basal ration. The further addition of nitrogen as ACM, therefore, would not be expected to produce as great a response as observed in trial 1, for, as pointed out by Maynard and Loosli (1956), as the protein content of the basal ration approaches the optimum level, each addition of protein (nitrogen) exerts less effect in this direction. As a consequence, most digestion coefficients (ether extract excepted) were increased only about 6 percentage units by the ACM supplement. As in trial 1, fecal excretion of dry matter, organic matter, and nitrogen-free extract was less on the supplemented ration than on the

basal, and digestion coefficients calculated by difference for these nutrients in the ACM were over 100%. Apparent digestibility of protein, so calculated, was 41.6%, which is in reasonable agreement with the value of 49.9% obtained in trial 1. Although higher digestibility values which approached 60% have been reported in previous work with steers (Tillman *et al.*, 1957), it is clearly evident that the nitrogen of this ACM product is less digestible than that of most common concentrate feeds of similar or even lower nitrogen content (Morrison, 1951).

The nitrogen data in table 2 show that the lambs were in negative nitrogen balance on both the basal and supplemented ration in trial 1, and in positive balance on both rations in trial 2. In trial 1, the supplement of ACM which added 3.41 gm. of nitrogen to the basal ration contributed toward a less negative nitrogen balance; it saved 1.20 gm. of nitrogen, the difference between the balance values of −2.32 (basal) and −1.12 (supplemented). On the questionable basis of calculation by difference, 35% (1.20/3.41 x 100) of the ACM nitrogen appeared to be utilized for this purpose. In trial 2, where both balances were positive and the corresponding rations were higher in nitrogen, only 15% of the ACM nitrogen was so utilized.

Trial 3. Sixty-four hours after the lambs were fed the high ACM rations one of them consuming the 33% ACM product became stimulated. A description of his response serves to show one of the two types of stimulation observed. When first noticed, the afflicted animal appeared to be in a daze and barely able to stay on his feet. He staggered for about 3 feet, struck a wall, fell down, got up, and then staggered 6 feet into another wall. At this time his hind legs gave way and he presented the appearance of sitting down to rest while he regained his senses. The entire period of stimulation lasted about 2 minutes. Immediately afterwards the lamb appeared normal and began eating. Twelve hours later a lamb receiving the 16% product performed in a similar manner. Within the 7-day test all animals at one time or another showed similar symptoms. Since the attacks were of short duration, it is probable that many cases of this type of stimulation were unobserved.

A second type of stimulation was first observed on the sixth day of the trial. At that time two lambs appeared dazed, and staggered about in the manner described above. After about 2 minutes they showed extreme nervousness. The pupils of their eyes became dilated. At this stage they ran about the stalls in a state of excitement and bleated almost constantly. They became belligerent and tried to run into the attendants. The afflicted lambs remained in this excited state for about 2 hours. The next day which was the last day of the trial, two other lambs became stimulated and showed the same response.

After the lambs had been fasted, they were again put on the ACM rations. It was not until about 60 hours later that any of the lambs showed stimulation. Within the next 3 days all of the animals fed ACM showed

at one time or another the mild form of stimulation. None of them showed the extreme form. The time interval of about 60 hours between initial feeding and development of symptoms suggests the active principle to be accumulative, and that a certain concentration is reached before outward symptoms appear. Control animals fed ordinary cane molasses in place of the ammoniated product behaved normally.

Trial 4. During the first 39 days of this trial lambs receiving the basal, ACM and soybean oil meal rations gained 0.09, 0.33 and 0.44 lb. daily. The next day one lamb on the basal ration died of pneumonia. At this time, two of the lambs fed ACM were dull and listless. These animals continued to eat as usual but stood with their heads down and gave the appearance of being in a stupor. Both of these died the next day. Post-mortem examination revealed that gastro-intestinal tract, kidneys, liver and other organs except the heart were normal as revealed by gross and histological examinations. Examination of the heart revealed a condition characterized by severe hemorrhage of the endocardium with involvement of the myo- and pericardium. The remaining ACM-fed sheep was slaughtered on the last day of the trial and examination of his heart also revealed hemorrhage in the endocardium with very slight involvement of the myocardium and no involvement of the pericardium. The remaining animals on the basal and soybean oil meal ration were slaughtered and gross examination revealed no abnormalities.

The presence in ACM of an imidazole-type derivative having pharmacological properties similar to histamine has been suggested by Pensack (1956). None of the clinical symptoms of histamine poisoning as described by Jones (1954) were observed in these animals.

Summary

The apparent digestibility of nitrogen in ammoniated cane molasses (ACM) was determined in two digestion and nitrogen balance trials with sheep. The ACM which contained the equivalent of 33% protein was added to low- and medium-protein basal rations in an amount which supplied 49% and 38%, respectively, of the total ration nitrogen. The apparent digestibility of the ACM nitrogen, calculated by difference, was 50% with the low protein basal and 42% with the medium-protein basal. Nitrogen balance was improved, although still negative, on the supplemented low-protein basal. Nitrogen retention was increased on the supplemented medium-protein ration.

Mild and extreme types of stimulation were produced by feeding high levels of these products. An ammoniated "high test" molasses in a fattening ration gave unfavorable results.

Literature Cited

Barrentine, B. F., and J. A. Darnell. 1954. Ammoniated molasses. Miss. Agr. Exp. Sta. Info. Sheet 498.

Briggs, H. M. and W. D. Gallup. 1949. Metabolism stalls for wethers and steers. J. Animal Sci. 8:479.

Davis, R. F., R. H. Wasserman, J. K. Loosli, and G. H. Grippin. 1955. Studies on the availability of nitrogen from various ammoniated products for rumen bacteria and dairy cattle. J. Dairy Sci. 38:677.

Forbes, E. B., R. W. Swift, J. W. Bratzler, Alex Black, E. J. Thacker, C. E. French, L. F. Marcy, R. F. Elliott and H. P. Moore. 1943. Conditions affecting the digestibility and metabolizable energy of feeds for cattle. Pa. Agr. Exp. Sta. Bul. 452.

Jones, L. M. 1954. Veterinary pharmacology and therapeutics. Iowa State College Press, Ames, Iowa.

Maynard, L. A. and J. K. Loosli. 1956. Animal Nutrition. 4th ed. McGraw-Hill Book Co., Inc. New York, New York.

Morrison, F. B. 1951. Feeds and Feeding. 21st ed. Morrison Publishing Co., Ithaca, New York.

Pensack, J. M. 1956. Commercial Solvents Corporation, Terre Haute, Indiana. Private Communication.

Richardson, D., E. F. Smith, F. H. Baker, R. F. Cox and K. L. McReynolds. 1954. The value of ammoniated molasses in beef cattle wintering rations. Kan. Agr. Exp. Sta. Circ. 308.

Stiles, H. R. 1952. Feed Supplement. U. S. Patent 2, 603, 567.

Tillman, Allen D., Willis D. Gallup, L. S. Pope, G. A. McLaren and W. Price. 1957. Utilization of ammoniated industrial products by cattle. J. Animal Sci. 16:179.

Tillman, A. D. and R. W. Swift. 1953. The utilization of ammoniated industrial by-products and urea by sheep. J. Animal Sci. 12:201.

Williams, V. J., M. C. Nottle, R. J. Moir and E. J. Underwood. 1953. Ruminal flora studies in the sheep: IV. The influence of varying dietary levels of protein and starch upon digestibility, nitrogen retention, and free microorganisms of the rumen. Australian J. Biol. Sci. 6:142.

SOME EFFECTS ON BEEF CATTLE OF PROTEIN AND ENERGY RESTRICTION

C. F. WINCHESTER, R. L. HINER, AND V. C. SCARBOROUGH [1]
U. S. Department of Agriculture

BEEF cattle possess potentialities for rapid growth that are seldom fully realized except in the production of baby beef. In contrast with the "continuous growth" of cattle on liberal rations like those used in the production of baby beef gains of a large proportion of range cattle are retarded during the fall and winter seasons, and in many cases gains are completely interrupted or the animals are subjected to actual loss of weight. Such retardation has been believed by many to result in later loss of at least part of the potentiality for rapid growth, of efficiency of feed utilization and of the potentiality for the production of high quality beef. None of these postulated harmful effects of retardation materialized during our earlier investigations with twin beef cattle (Winchester *et al.*, 1954, 1955, 1957) in which the retarded animals were given liberal allowances of all of the nutrients known to be required by cattle with the exception of energy sufficient for rapid gains. While interruption of weight gains apparently was not followed by unfavorable results in the earlier experiments, it is possible that harmful effects might accompany restriction simultaneously of energy and an essential nutrient such as protein. The present experiment was designed to test the effects of such restriction. Protein intake was limited, together with that of energy, in preference to restriction of some other essential nutrient because the protein requirement is one that ordinarily can be met under winter range conditions only at a sizeable monetary outlay.

Procedure

The animals used in these trials were identical twin, beef-type cattle. Determination of the probable monozygotic origin of the twins was made by critical physical examinations which later were confirmed by blood tests of an antigenic type.[2] Individuals were slaughtered on reaching either the low prime grade or the highest grade below low prime that the animal reasonably could be expected to attain. The breeds and crosses represented are given by table 1. One member of each of 2 cross-bred pairs died prior to time of slaughter leaving surviving co-twins that remained as experimental animals throughout the trials. Some data on animal 41, a crossbred steer that died about a month before its co-twin was slaughtered, have been

[1] Animal Husbandry Research Division, A. R. S., Beltsville, Md.

[2] Grateful acknowledgment is made of the assistance given by M. R. Irwin *et al.*, Department of Genetics, University of Wisconsin, in whose laboratory the blood samples were analyzed.

tabulated. The quarters occupied by the animals and details of their care were described earlier (Winchester and Howe, 1955).

Eight different rations were used; the TDN of each was 67% (dry matter basis). Protein, fiber, N.F.E. and fat content of the pelleted rations were determined by chemical analysis and each constituent was multiplied

TABLE 1. FEED UTILIZATION DURING RESTRICTION

Animal No.	Breed or cross [a]	Ration	Percentage of liberal rations consumed during restriction [c]		Daily weight gain	Length of period	Feed efficiency [b] per pound of gain		
			Protein	Energy			Digestible energy	TDN	Digestible protein
	sex [a]	No.	%	%	lb.	days	therms	lb.	lb.
59	s	1	13	42	—0.13	196	....	....	....
60	A	7	76	86	1.55		8.0	4.3	0.46
46	s	1	14	44	—0.13	179	....	....	....
45	A	2	46	42	0.11		....	....	....
50	s	1[d]	21	37	—0.08	182	....	....	....
49	H x G	6	116	68	1.03		10.0	4.9	1.00
47	f	2	44	40	0.16	181	....	....	....
48	A	3	67	38	0.42		....	....	....
40	s	4	65	72	1.16	189	9.9	5.3	0.58
39	MS	5	96	73	1.42		7.9	4.2	0.67
56	f	4	65	69	1.21	185	9.3	4.8	0.55
55	A	3	69	42	0.32		....	....	....
44	f H x J	4	67	70	1.25	189	8.5	4.4	0.50
53	f	3	69	44	0.47	182	....	....	....
54	A x J	6	118	78	1.25		7.9	3.9	0.78
57	f	4	61	62	1.22	175	8.5	4.4	0.50
58	H x G	8	89	56	1.12		8.4	4.2	0.78
61	f	7	80	94	1.86	202	8.7	4.9	0.50
62	MS	5	82	68	1.02		10.5	5.8	0.84
41	s	5	102	73	1.25	187	10.7	5.4	0.94
42	H x A	6	127	72	1.00		12.7	6.5	1.40
52	f	7	87	93	1.37	182	9.9	5.1	0.57
51	A	8	132	88	1.34		9.7	4.9	0.88

[a] Meanings of letters are as follows: s, steer; f, female; A, Aberdeen Angus; G, Guernsey; H, Hereford; J, Jersey; MS, Milking Shorthorn.

[b] Animals fed above maintenance level.

[c] Protein and energy, expressed as percentages of liberal allowances fed between the ages of 6 and 12 months. Requirements are assumed to vary with the 0.7 power of body weight. Liberal allowance of a 400-lb. animal is 0.9 lb. of digestible protein, and 14.7 therms of digestible energy or 7.4 lb. of TDN daily.

[d] Animal refused ration 1 after consuming it for 4 months and thereafter ate only ration 2.

by a factor equal to a composite value representing its digestibility in the given ration as calculated on the basis of digestibility data given by Morrison (1956). For example, the digestible protein of ration 7 is contained by the alfalfa meal and corn (table 2). The ration contains 9.8 lb. of crude protein and 6.5 lb. of digestible protein and therefore the calculated coefficient of digestibility is 6.5/9.8 or 66%. Digestible energy in therms was calculated as the sum of kg. digestible protein X 5.65 plus kg. digestible fat X 9.3 plus kg. digestible carbohydrate X 4.1.

Three of the eight rations provided for caloric maintenance, three for a pound of daily gain, and two for 2 lb. of gain each day while protein levels provided by different rations of like caloric value were quite different as is shown by tables 1 and 2. All rations were fed in pellet form during restriction, and following restriction hay and grain rations were used. Our criteria of a liberal ration are the protein allowance recommended by a committee of the National Research Council (Guilbert *et al.*, 1950) for

TABLE 2. RATIONS USED IN THESE TRIALS [a]

Ration No.	1	2	3	4	5	6	7	8
Energy level	Maintenance			1 lb. potential gain [b]			2 lb. potential gain [b]	
Digestible protein, %	2.4	6.5	12.4	6.7	10.4	13.7	6.5	11.4
Daily allowance 400-lb. calf (lb.)	5.1	5.2	5.1	8.9	8.8	9.0	13.1	13.2
Constituents, parts by weight								
Alfalfa meal	3.6	33.9	74.3	33.0	59.1	74.3	32.3	60.6
Corn cobs	28.5	17.3	....	33.0	7.9	....	34.6	13.4
Corn meal	14.2	15.1	22.8	31.9	31.2	18.6	31.7	20.1
Corn starch	14.2	8.7	....	....	....	....	....	....
Hay, rough, ground	26.8	16.4	....	....	....	....	....	....
Linseed oil meal	....	....	....	....	....	5.5	....	4.4
Molasses	8.8	5.4	....	....	....	....	....	....
Bone meal, steamed	1.9	0.8	....	0.2	....	....	....	....
Monosodium phosphate	1.0	1.4	1.8	0.9	0.8	0.6	0.4	0.5
Salt	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total	100.0	100.0	99.9	100.0	100.0	100.0	100.0	100.0

[a] These rations were used during restricted feeding only. TDN was 67% of dry matter of each ration; all were fed as 5/8 inch pellets.
[b] Daily gain in body weight.

400-lb. beef calves growing at a normal rate, and the level of energy we used earlier to represent a liberal ration (Winchester *et al.*, 1954, 1955, 1957), namely 0.9 lb. of digestible protein and 7.4 lb. TDN daily for a 400-lb. animal. Both protein and energy allowances varied with the 0.7 power of body weight.[3]

The experimental design is of a quasi factorial type, providing for 8 treatments when 12 pairs of identical twins are used, described by Dick and Whittle (1951). This design provides for the feeding of rations 1 to 8 to

[3] Winchester and Hendricks (1953) found that the energy requirements of 16 pairs of identical twin calves varied with the 0.6686 power of body weight. We have used a single integer to express the power of body weight, in our application of this finding, in recognition of the fact that the exponent may not be significant beyond the first integer following the decimal.

TABLE 3. FEED UTILIZATION DURING RECOVERY AND ENTIRE TRIAL

Animal No.	Ration No.	Recovery phase					Entire trial				
				Feed efficiency per lb. of gain					Feed efficiency per lb. of gain		
		Length	Daily gain	Digestible energy	T.D.N.	Digestible protein	Length	Daily gain	Digestible energy	T.D.N.	Digestible protein
		days	lb.	therms	lb.	lb.	days	lb.	therms	lb.	lb.
59	1	454	1.80	11.8	5.7	0.71	650	1.22	12.5	6.4	0.76
60	7	350	1.37	16.0	8.2	1.04	546	1.43	12.9	6.6	0.81
46	1	419	1.63	12.2	6.3	0.79	598	1.09	14.0	7.2	0.85
45	2	419	1.53	13.3	6.8	0.86	598	1.10	14.3	7.4	0.93
50	1	496	1.58	13.0	6.7	0.83	678	1.14	14.3	7.4	0.88
49	6	406	1.49	15.1	7.8	0.96	588	1.35	13.9	7.1	0.97
47	2	378	1.57	12.9	6.6	0.82	559	1.11	13.9	7.1	0.88
48	3	378	1.48	13.5	6.9	0.86	559	1.14	13.4	6.9	0.91
40	4	424	1.69	13.7	7.0	0.85	613	1.52	12.8	6.6	0.79
39	5	461	1.58	15.0	7.7	0.93	650	1.54	13.1	6.7	0.86
56	4	406	1.22	19.4	10.0	1.22	591	1.22	16.3	8.4	1.01
55	3	461	1.47	15.5	8.0	0.95	646	1.14	15.7	8.1	1.02
44	4	328	0.98	17.0	8.7	1.17	517	1.08	13.4	6.9	0.89
53	3	419	1.44	13.1	6.7	0.83	601	1.15	12.8	6.6	0.86
54	6	384	1.20	16.5	8.5	1.04	566	1.22	13.6	7.0	0.96
57	4	363	0.94	19.0	9.7	1.22	538	1.03	15.0	7.7	0.94
58	8	363	0.98	17.8	9.4	1.16	538	1.04	14.5	7.6	1.03
61	7	316	1.64	14.2	7.3	0.87	518	1.72	11.9	6.1	0.71
62	5	469	1.40	17.6	9.1	1.07	671	1.28	15.9	8.2	1.01
41	5	372	1.33	15.6	7.3	1.02	559	1.30	14.0	7.2	1.00
42	6	398	1.39	14.4	9.6	0.97	585	1.20	14.7	7.7	1.13
52	7	266	1.24	17.2	8.8	1.10	448	1.29	14.0	7.2	0.87
51	8	266	1.33	15.8	8.0	1.03	448	1.33	13.3	6.8	0.97

the pairs of twins as follows: 1 vs. 2, 1 vs. 6, 1 vs. 7, 2 vs. 3, 2 vs. 8, 3 vs. 4, 3 vs. 6, 4 vs. 5, 4 vs. 8, 5 vs. 6, 5 vs. 7, and 7 vs. 8. As table 1 shows, the only pair of rations not fed during the experiment was 2 vs. 8; this resulted from the fact that one of the animals given this paired treatment died at an early age while its co-twin inadvertently became pregnant during the trials. No. 44, the co-twin of another animal that died at an early age, was fed ration 4.

The animals were fed restricted rations between the ages of 6 and 12 months. After restriction ended, each animal was allowed as much of a good growing ration as it could consume readily.

Results

As shown in table 3, digestible energy consumed per lb. of gain within pairs, from age 6 months until slaughter, was quite similar. With one exception the within pair difference was only 2 to 6% while the difference between means of pairs was, in some cases, much greater. For example, animal 59 received only 13% of a liberal protein allowance and only 42% of a liberal energy allowance while its co-twin was fed 76 and 86%, respectively, of liberal protein and energy allowances during the period of restriction (table 1). In spite of the wide differences in treatment during the period between the ages of 6 and 12 months, the difference in digestible energy per lb. of gain was only about 3%. Similarly, animal 50 was highly restricted both as to protein and caloric intake while its co-twin received a liberal protein allowance and energy sufficient for a daily gain of one pound. While the within pair differences in digestible energy per unit gain are in both these pairs about 3%, the difference between the means of the two pairs is around 10%. Co-twins 61 and 62, unlike the other pairs, exhibited a relatively wide within-pair difference in feed efficiency that appears to have been due to the slow growth of 62 following feed restriction. While this could have been due to a condition of impaired health of the individual, no evidence of a pathological condition was observed during or after the trial. Another possible explanation is that these co-twins may not have been identical; nevertheless, by the criteria available to us, this pair was judged probably identical.

Some carcass data are given by table 4. Animal 59, one that received ration 1, the allowance that, of the 8 used during restriction, was of least protein value, possessed a slightly smaller percentage of muscle and a slightly greater percentage of fat than did its continusuoly well-fed co-twin. More conspicuous within pair differences were shown by 49 and 50; The latter consumed ration 1 for 4 months and then was given ration 2 because it refused ration 1. This suggests that extremely severe protein and energy restriction, together, might influence carcass composition adversely. Aside from the differences within these two pairs that included animals fed ration 1, the data do not suggest that restriction for 6 months measurably influ-

TABLE 4. QUALITY AND SIZE OF CARCASSES

TABLE 4. QUALITY AND SIZE OF CARCASSES

Animal No.	Ration No.	Carcass				Dressing percentage [b]	Body weight [c]	Body measurements [d]		
		Grade	Composition [a]					Height at withers	Length of body	Circumference at foreflank
			Muscle	Fat	Bone					
			%	%	%	%	lb.	cm.	cm.	cm.
59	1	High good	52.4	31.3	16.3	65	1061	120	136	189
60	7	Medium choice	55.2	30.3	14.4	65	1051	118	138	191
46	1	Medium choice	52.4	34.1	13.5	65	961	114	135	182
45	2	Medium choice	51.4	34.9	13.7	67	963	114	136	186
50	1	High choice	51.3	34.7	14.0	61	1059	126	137	189
49	6	Medium good	55.0	29.2	15.8	64	1083	125	147	191
47	2	Low choice	43.8	43.7	12.5	66	944	113	130	180
48	3	Low choice	44.3	42.8	13.0	65	949	113	130	181
40	4	Low good	51.7	33.4	14.9	63	1311	128	141	198
39	5	Low good	48.3	37.1	14.6	64	1316	132	156	199
56	4	Low choice	53.3	32.3	14.4	64	1083	120	138	189
55	3	Medium choice	51.9	34.9	13.1	62	1085	118	139	192
44	4	Medium good	51.8	33.3	14.9	65	864	116	130	171
53	3	High good	51.3	34.5	14.2	60	931	112	137	180
54	6	Medium good	48.9	36.9	14.2	61	924	114	138	179
57	4	High commercial	56.3	26.2	17.5	59	905	119	142	174
58	8	High commercial	55.8	28.6	15.6	60	913	119	143	176
61	7	High commercial	48.3	36.2	15.4	65	1279	131	147	196
62	5	High commercial	55.3	26.7	18.1	64	1246	133	162	195
41 [b]	5	—	—	—	—	—[e]	1174	—	—	—
42	6	Low good	54.2	28.7	17.1	60[f]	1141	127	156	196
52	7	Medium choice	51.8	34.4	13.8	65	853	111	126	178
51	8	Medium choice	52.8	33.4	13.8	64	879	111	128	183

[a] Equations derived by Hankins and Howe (1946) have been used to estimate the physical composition of dressed carcasses. The equations are as follows:
Separable muscle of dressed carcass=16.08+0.8 percentage of separable muscle in 9th-10th-11th-rib cut.
Separable fat of dressed carcass=3.54+0.8 of percentage of separable fat in 9th-10th-11th-rib cut.
Separable bone and tendon of dressed carcass=5.52+0.57 of percentage of separable bone and tendon in 9th-10th-11th-rib cut.

[b] Dressing percentage equals $\frac{\text{cold weight}}{\text{final weight in feed lot}} \times 100$

[c] Final weight in feed lot.

[d] At time of slaughter.

[e] No. 41 died, apparently due to severe ulceration of colon. After slaughter, No. 42 was found to be similarly, but less severely, ulcerated.

enced the carcass composition. A few categories of observations of meat quality from among the many that were made are given by table 5. The results of these tests suggest that early nutritional restrictions did not influence the desirability of flavor of the *lean meat* adversely. On the other hand, flavor of the *fat* of animals 50 and 59, fed ration 1, was in each case judged less desirable than the fat of its well-fed co-twin. This suggests

TABLE 5. MEAT QUALITY [a]

Animal No.	Ration No.	Tenderness	Desirability of flavor		Area of eye of rib cut
			Fat	Lean meat	
					sq. in.
59	1	5.4	4.8	6.0	12.0
60	7	5.8	5.4	5.8	11.6
46	1	6.4	5.8	6.4	12.6
45	2	5.8	5.8	5.4	11.6
50	1	5.6	5.2	6.0	10.5
49	6	5.4	6.0	6.6	13.7
47	2	6.0	5.0	6.2	11.5
48	3	5.6	5.6	5.8	9.7
40	4	5.8	5.6	6.0	11.6
39	5	5.2	5.8	5.6	9.6
56	4	5.0	5.6	6.4	12.3
55	3	6.0	5.8	6.2	11.7
44	4	5.4	6.0	6.4	12.0
53	3	6.4	6.4	6.6	11.0
54	6	6.4	6.2	6.4	11.1
57	4	4.8	6.2	6.4	10.1
58	8	5.4	5.8	5.8	10.2
61	7	4.6	5.4	5.6	10.5
62	5	6.0	5.8	6.0	12.9
41	5	—[b]	—	—	—
42	6	6.2	5.4	6.2	9.8
52	7	—	—	—	12.3
51	8	—	—	—	11.9

[a] The numbers 1 to 7 indicate the degree either of tenderness or desirability of flavor. Number 1 indicates that a desirable factor cannot be perceived, number 7 that the meat is extremely tender or that the flavor is excellent, depending on the column in which the digit is found.

[b] Animal 41 died, apparently due to severe ulceration of colon. After slaughter, 42 was found to be similarly, but less severely, ulcerated.

that flavor of fat may be influenced by severe protein and energy restrictions.

As is shown by table 1, most of the animals fed rations 7 and 8, allowances providing energy for 2 lb. of possible gain daily, failed to make the expected gains. This resulted mostly from the fact that the animals failed to consume all the feed offered them. Feed efficiency on these rations was not below that on the other rations as table 1 shows. For example, No. 58 fed ration 8, gained less than its co-twin given ration 4, but feed efficiency of the two animals was nearly alike.

The effects of the experimental treatments on efficiency of feed utilization and quality of carcasses and meat are not, in our judgment, great enough to justify application of the statistical treatment called for by the experimental design. However, data obtained in this experiment together with additional data recorded in other growth trials will be subjected to comprehensive statistical analyses at an early date. While the treatments did have but little influence upon over all feed efficiency and quality of

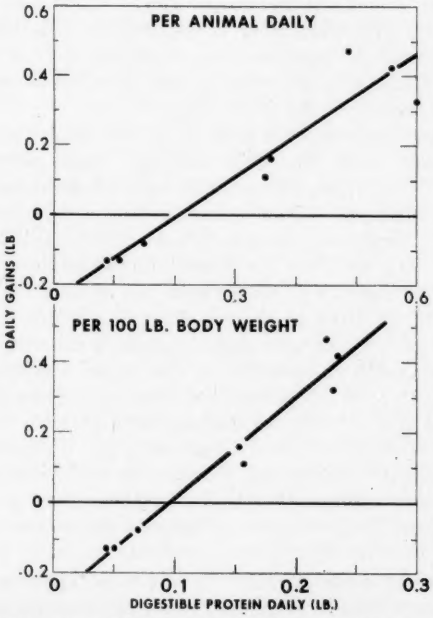

Figure 1. Relationship between protein intake of calves on caloric maintenance rations and changes in body weight. Shown in the lower part of the figure is a fitted regression line having the equation: $Y = -0.2723 + 2.8510X$. The 0.7 power of body weight was used in calculating protein intake per 100 lb. of body weight.

the products, table 3 shows that individuals given the highly restricted rations reached market grade later than did their well-fed co-twins.

Of the animals fed at approximately the level of caloric maintenance, those given the ration lowest in protein lost weight while those fed the two other rations made small gains. These bodyweight changes formed a definite pattern as is shown by figure 1. The upper part of the figure gives the protein intake of individuals; the lower, the relationship between gains and protein intake per 100 lb. unit of body weight when the calves were on

caloric maintenance rations. The 0.7 power of bodyweight was used in relating daily protein intake to the 100 lb. unit of bodyweight. As the figure shows, calves lost weight when digestible protein per 100 lb. of bodyweight was from 0.04 to 0.07 lb. per day but gained when they received 0.15 to 0.24 lb. daily. The fact that differences, as great as 0.43 therm, existed in caloric intake per 100 lb. of body weight led us to make a correction for energy intake. This correction consisted of the calculation of the probable change in body weight if energy intake of each animal were equal to the mean intake of the group, namely 2.27 therms per 100 lb. of bodyweight daily. This relationship is represented by the lower line shown in figure 1. If Y of the equation given with the figure is made equal to zero (absence of change in bodyweight), the digestible protein ingested per 100 lb. of bodyweight is about 0.1 lb. daily.[4]

When a comparison is made of some of our results of feeding low protein rations with earlier work done with dairy cows, some interesting parallels come to light. Nitrogen equilibrium has been reported in dairy cows at daily levels of digestible protein intake as low as 0.21 and 0.25 lb. per 1,000 lb. of body weight according to Armsby (1917). If protein utilization is assumed to vary with the 0.7 power of bodyweight, these levels are equivalent to 0.04 and 0.05 lb. for a 100 lb. calf or about the level provided by ration 1. On the basis of the information available to him, Armsby concluded that a 1,000 lb. cow should receive a minimum of 0.6 lb. of digestible protein daily. However, in arriving at this conclusion he excluded the 0.21 and 0.25 levels of protein and used an average of more liberal allowance. Hills *et al.* (1922), on the basis of a relatively large number of data agreed that Armsby's level is adequate for maintenance. Mitchell (1929) subsequently pointed out that it not only provides protein for maintenance but in addition supplies a liberal margin of safety. This same level was accepted by Loosli *et al.* (1950) as the maintenance allowance for dairy cows.

One of the present needs in cattle feeding is an index of the minimum practical level of protein to be allowed young cattle on caloric maintenance rations. We suggest that the present level of 0.6 lb. digestible protein per 1,000 lb. for dairy cows on maintenance rations can be extrapolated safely, if allowances are varied with the 0.7 power of bodyweight, to provide for young cattle on caloric maintenance rations. For animals of 100, 200, 400, 600 and 800 lb. weight equivalent allowances are 0.12, 0.19, 0.31, 0.42, and 0.51 lb. of digestible protein daily. Such an allowance furnishes over twice the level of protein of our ration 1, about ¾ as much as our ration 2, or 1¼ times the level at which the fitted regression line of figure 1 intersects the line representing zero gain. This intersection point, in the sense that it represents a protein level that would result in maintenance of bodyweight when the mean energy level provided the animals of figure

[4] Grateful acknowledgment is made of the assistance of M. J. Morris, Biometrical Services, A.R.S., who made these computations.

1 is fed, can be considered to be a hypothetical level of protein for maintenance. Beyond doubt further work will show what interactions take place between protein and energy at and near the level of maintenance. Our suggestion concerning protein allowances is made merely to fill a practical need, on a provisional basis, until further pertinent information can be obtained.

Summary

The effects of rations of low protein as well as low energy value, fed between the ages of 6 months and one year, were studied with monozygotic beef-type cattle. The experiments were begun with 12 pairs of animals of which 10 pairs remained intact throughout the trials. Eight different pelleted rations were fed, ranging from a caloric maintenance ration that was only 2.5% digestible protein (ration 1) to rations of relatively high energy value liberally supplied with protein. During restriction, animals on ration 1 lost weight while those on the other rations made gains that ranged from slight increases to 1.9 lb. a day depending on the quality of the rations and the amounts fed. Slaughter of each retarded animal was delayed until its degree of fatness approximated the final condition of its co-twin; slaughter weights of co-twins were similar. In spite of the drastic treatments given some animals, efficiency of feed utilization and carcass and meat quality of co-twins, with one partial exception, were similar. The exception consisted of a lower degree of desirability of fat of 2 of the animals fed ration 1 as compared with the fat of their co-twins. Within one of the pairs this was likewise true of the lean meat while within the other pair it was not. When energy intake was held near maintenance, changes in bodyweight were positively correlated with level of protein intake. The existing need for an index of the minimum practical level of protein to be allowed young cattle on caloric maintenance rations is discussed and an allowance to meet this need is suggested on a tentative basis.

Literature Cited

Armsby, Henry Prentiss. 1917. The Nutrition of Farm Animals. The Macmillan Co., N. Y.

Dick, I. D. and P. Whittle. 1951. Contributions to the statistical design of twin experiments. Part I. Twin designs and their efficiency. New Zealand J. Sci. and Tech. 33: Sec. B 145.

Guilbert, H. R., Paul Gerlaugh and L. L. Madsen. 1950. Recommended nutrient allowances for beef cattle. National Research Council, No. IV.

Hankins, O. G. and Paul E. Howe. 1946. Estimation of the composition of beef carcasses and cuts. U. S. Dept. Agr. Tech. Bul. 926.

Hills, J. L., C. L. Beach, A. A. Borland, R. M. Washburn, G. F. E. Story and C. H. Jones. 1922. The protein requirements of dairy cows. Vt. Agr. Exp. Sta. Bul. 225.

Loosli, J. K., C. F. Huffman, W. E. Petersen and P. H. Phillips. 1950. Recommended nutrient allowances for dairy cattle. National Research Council. No. III.

Mitchell, H. H. 1929. The minimum protein requirements of cattle. National Research Council Bul. No. 67.

Morrison, F. B. 1956. Feeds and Feeding. The Morrison Publishing Co., Ithaca, N. Y.

Winchester, C. F. and Walter A. Hendricks. 1953. Energy requirements of beef calves for maintenance and growth. U. S. Dept. Agr. Tech. Bul. 1071.

Winchester, C. F. and Paul E. Howe. 1954. Some effects on beef cattle of interrupted growth I and II. U. S. Dept. Agr. Mimeo Information Pamphlets.

Winchester, C. F. and Paul E. Howe. 1955. Relative effects of continuous and interrupted growth on beef steers. U. S. Dept. Agr. Tech. Bul. 1108.

Winchester, C. F. and N. R. Ellis. 1957. Delayed growth of beef cattle. U. S. Dept. Agr. Tech. Bul. 1159.

THE BIOLOGICAL AVAILABILITY OF CALCIUM IN FEEDS FOR CATTLE [1]

SAM L. HANSARD,[2] H. M. CROWDER AND W. A. LYKE

University of Tennessee—Atomic Energy Commission, Agricultural Research Program, Oak Ridge, Tennessee

THE value of a feed as a source of calcium depends not only upon the calcium content, but also on the amount that the animal can extract and retain for its own use. There have been variations in procedures used for the measurement of calcium availability for the various species. Whole body analysis (Drake *et al.*, 1949; Sur and Subrahmanyan, 1952) or bone ash values (Armstrong and Thomas, 1952) have been customary methods when rats were employed as experimental animals. However, conventional balance trials have been routinely used in calcium utilization studies with man (Mitchell and Curzon, 1939) and with farm animals (Forbes *et al.*, 1929; Lindsey and Archibald, 1925; Ellenberger *et al.*, 1931, 1932; Weber, 1940). These results have supplied nutritionists with much valuable basic information on the biological availability of calcium from the various dietary sources. Bone breaking strength, milk production, growth data and blood values have been used as supporting criteria for determining the calcium status of animals in relation to dietary treatment (Moxon *et al.*, 1947; Lantow, 1933). Comparative availability studies of calcium sources with cattle, however, have been limited, necessitating much extrapolation of utilization data from the results of studies with other species.

Advances in radioisotope procedures have permitted the quantitative differentiation between that portion of the dietary calcium that passes unabsorbed through the gastrointestinal tract and the metabolized excreted calcium, thereby making possible a simple and direct means for the quantitative separate estimation of the endogenous and exogenous calcium fractions in the feces of the normal intact animal. This has made feasible the subsequent independent measurement of the *true* absorption of dietary calcium (Hansard *et al.*, 1954). This paper presents the results of concurrent chemical and radiocalcium balance studies under controlled conditions which reflect the endogenous loss and permits the calculation of calcium availability from selected sources of organic and inorganic calcium fed to young and mature cattle.

[1] This manuscript is published with the permission of the Director of the University of Tennessee Agricultural Experiment Station, Knoxville, Tennessee.

[2] Present address: Animal Industry Department, L.S.U., Baton Rouge, La.

The Radioactive materials employed were obtained from the Oak Ridge National Laboratory on allocation from the United States Atomic Energy Commission.

This study was completed under Contract No. AT–40–1–GEN–242 between the University of Tennessee, College of Agriculture, and the Atomic Energy Commission.

Experimental Procedures

Mature Hereford steers and Hereford calves 5 to 7 months of age were pair-fed the basal concentrate mixture shown in table 1, supplemented as indicated with the various calcium sources (table 2). The minerals to be tested were calculated to contribute the greater part of 12 and 20 gm. of calcium daily for the young and mature animals, respectively, and were substituted in the basal concentrate mixture at the expense of the ground yellow corn. Dietary phosphorus was maintained at approximately 10 and 18 gm. daily for the young and mature animals, respectively, by added supplements of sodium phosphate when necessary as determined by analysis. The calves received 2 lb. of orchard grass hay and 6 lb. of the concentrate mixture daily, whereas, the mature steers received an average of 5 and 12 lb., respectively. In those studies where calcium availability of the hays was being determined, hay intake was increased to the necessary level to supply the desired dietary calcium, at the expense of the basal concentrate mixture. Animals were maintained throughout the experiment

TABLE 1. PERCENTAGE COMPOSITION OF CONCENTRATE FED

	Mature steers	5–7 month calves
Corn, yellow	65.0	68.0
Oats, crushed	21.7	22.4
Cottonseed oil meal	12.4	9.0
Yeast [a]	.01	.01
Mineral supplement [b]	0.9	0.6

[a] Added to supply 1500 I.U. Vitamin D.
[b] Calculated to provide a total of 20 and 12 gm. of calcium per day for mature and young steers respectively.

on the same basal concentrate mixture to facilitate relative comparison of the added calcium supplements. The level of feeding was established 10 to 20 days prior to and remained unchanged throughout each experimental balance period. The general procedures for management of the animals have been discussed by Hansard *et al.* (1951) and analytical methods employed have been described by Comar *et al.* (1951).

Measurement of Calcium Availability. It has been shown in previous reports that the *true* absorption of calcium in cattle feeds can be calculated from results of conventional balance studies when the endogenous fecal calcium losses are known. The comparative balance method of Hansard *et al.* (1954) and the isotope dilution procedure described by Visek *et al.* (1953) were used concurrently to measure the fecal endogenous losses in these studies. The latter method was modified to include a single intravenous calcium-45 dose and a 7-day balance rather than the more tedious 10-day radiocalcium injection period (Hansard, 1956). Endogenous fecal

calcium (E) was then determined from the average specific activity (SA) values for the feces and plasma on the last two days of the balance trial, thus:

$$E=\frac{SA_f}{SA_p} \times \text{Daily fecal calcium.}$$

The comparative balance procedure is unique for biological availability studies in that it permits either a direct measurement of absorption or the estimation of endogenous calcium losses for use in correcting the conventional balance to give the *true* absorption values. The 7-day excretion data from animals dosed orally and that of paired animals receiving a single intravenous dose of labeled calcium permitted calculation of the percentage of dietary calcium that was absorbed (A) thus:

$$A=\frac{100-\text{Fecal calcium}-45}{1-B}$$

where (B) represents the fraction of an intravenous dose appearing in the feces. The percentage of endogenous fecal calcium (E) was then calculated (Hansard, 1956) as:

$$E=A-(100-\text{Percent fecal calcium}).$$

The daily endogenous fecal calcium in grams was obtained by multiplying E by the total dietary calcium intake and dividing by 100. Briefly, therefore, the biological availability of calcium from the specific dietary sources reported was measured as follows: 1) A ration was formulated that was nutritionally adequate but low in calcium. 2) The supplement was incorporated to supply minimal calcium requirements, yet permitting maximal contribution from the mineral additive. 3) Feeding level for the ration was established several weeks before, and level of feeding maintained throughout the experimental period. 4) Endogenous calcium was measured immediately preceding or concurrently with conventional balance studies, and 5) The balance data were corrected for endogenous calcium losses to give the true digestibility (TD) values thus:

$$TD=100-\frac{(\text{Fecal calcium minus endogenous calcium})}{\text{Calcium intake}}\ 100$$

Results and Discussion

A summary of the results from 109 concurrent chemical and radiocalcium balance studies with young growing steers and mature cattle fed some common sources of dietary calcium are shown in table 2. It is noted that the high endogenous calcium loss in the mature steers tended to mask the *true* digestibility values, indicating poor absorption many times when net retention from the source was actually high. In the young growing calves apparent digestibility more nearly approached the *true* absorption values, a reflection of the greater retention and lower endogenous losses. These studies and earlier work by Hansard *et al.* (1954) indicate that endogenous

TABLE 2. SUMMARY OF BIOLOGICAL AVAILABILITY STUDIES WITH YOUNG AND MATURE STEERS FED VARIOUS AND INORGANIC SOURCES OF CALCIUM

Calcium source	Number of animals	Average weight	Calcium		Endogenous[a] calcium	Digestibility	
			Intake	Excretion		Apparent[b]	True[c]
		lb.	gm./day		gm./day		
Mature steers							
Bone meal (imported)	4	847	15.5	13.1	6.1	16	55
Calcium carbonate (C.P.)	6	856	15.0±2	14.8	5.8±2.0	1	40
Calcium chloride (C.P.)	6	853	20.0±3	14.8	5.3±1.5	26	53
Calcium phosphate (Di-basic C.P.)	2	953	19.1	16.5	6.9	14	50
Calcium phosphate (Monobasic C.P.)	2	967	18.9	15.4	7.0	19	56
Dicalcium phosphate (A)	2	1295	20.9	20.2	9.4	4	49
Dicalcium phosphate (B)	2	1263	16.0	19.0	9.1	—19	38
Dicalcium phosphate (C)	4	1241	18.0	17.6	9.6	2	56
Dicalcium phosphate (D)	2	1335	17.1	18.0	9.7	—5	51
Dicalcium phosphate (E)	2	1272	18.6	17.8	9.5	4	55
Dicalcium phosphate (Defluorinated com.)	2	1250	19.5	20.8	9.1	—7	40
Ground limestone (com.)	6	790	21.1±2	18.0	4.7±1.1	15	37
Alfalfa hay #3	6	1108	23.8±3	24.0	7.6±1.6	—1	31
Lespedeza hay #2	4	991	24.5	22.5	6.7	8	36
Orchard grass #2	4	1017	15.0	16	6.8	—7	39
Young steers							
Bone meal (imported)	4	400	12.0	6.4	2.6	47	68
Calcium carbonate (C.P.)	6	486	11.9±2	8.8	2.9±0.4	26	51
Calcium chloride (C.P.)	4	397	12.2±3	7.6	2.7±0.6	38	60
Calcium phosphate (Di-basic C.P.)	2	500	14.0	8.5	3.4	39	64
Calcium phosphate (Monobasic C.P.)	2	505	13.3	8.7	3.5	35	61
Dicalcium phosphate (A)	2	344	10.5	6.7	2.3	36	58
Dicalcium phosphate (B)	2	332	8.1	5.9	2.4	27	56
Dicalcium phosphate (C)	3	323	8.5	5.6	2.2	44	60
Dicalcium phosphate (D)	2	392	6.9	5.5	2.7	20	60
Dicalcium phosphate (E)	2	409	7.9	6.1	2.8	23	58
Dicalcium phosphate (Defluorinated com.)	2	321	8.3	5.9	2.2	29	55
Ground limestone (com.)	6	345	14.4±3	10.4	2.5±0.5	28	45
Alfalfa hay #3	6	339	9.7±4	8.0	2.3±0.4	18	41
Lespedeza hay #2	4	535	11.0	9.1	3.6	17	50
Orchard grass #2	4	557	10.0	8.4	3.5	26	51

[a] Calculated from average results of isotope dilution and comparative balance procedures.

[b] Calculated as $100\left(\frac{\text{Ca intake} - \text{fecal Ca}}{\text{Ca intake}}\right)$.

[c] Calculated as $100 - \frac{(\text{Ca excreted} - \text{endogenous fecal Ca})\ 100}{\text{Ca intake}}$.

calcium losses tended to be slightly higher for old than young growing cattle, but approximated 16 mg. per kilogram body weight at this calcium status. For practical purposes, therefore, where facilities are not adequate for radioisotope studies this correction could be applied to advantage to

the results of conventional balance studies with cattle on standardized rations to approximate the biological values.

A survey of the *true* digestibility values for 15 selected calcium sources are graphically presented in figure 1. It is of interest that age of animal appeared to have a greater effect upon absorption than did source of the feed calcium. Availability from inorganic calcium sources tended to be somewhat higher than from alfalfa, lespedeza or orchard grass hays. This may have been due in part to a lower dietary protein level (Miller, 1926; Hart *et al.*, 1922), solubility, or to the presence in hays of calcium-binding substances that decreased availability for absorption. A slightly higher fecal

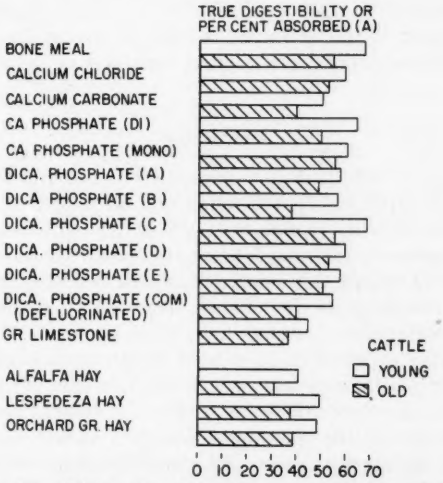

Figure 1. True digestibility of calcium from various organic and inorganic sources fed to young and mature steers.

excretion of radiocalcium following intravenous administration to alfalfa-fed cattle indicated a marked tendency toward an increased endogenous calcium loss. However, more animals would probably be required to show the significance of this trend. The lack of agreement between the *true* digestibility values for alfalfa which was fed at 2 to 4 lb. per day and orchard grass fed at 8 to 10 lb. per day would indicate that ration bulk was not the contributing factor. In general, however, the differences in calcium availability for cattle from the various sources were not great. These differences due to source were somewhat more apparent in mature than in young growing animals. However, availability values for calcium phosphate, bone meal and the dicalcium phosphate rated highest for both age groups. Ground limestone, defluorinated calcium phosphate and calcium carbonate were intermediate between bone meal and the hay sources.

This is in agreement with the work of Turner *et al.* (1927), Lindsey and Archibald (1925) and Hayden *et al.* (1930) who reviewed some of the early work with cattle and reported dicalcium phosphate to be as good a source of calcium as that from natural sources. The results of 27 balance studies with both young and mature cattle indicated no significant differences in calcium availability from the five commercial sources (A, B, C, etc.) of dicalcium phosphate fed in these studies. Using body-weight gains, Lantow (1933) noted little difference between rations containing monocalcium phosphate, bone meal, dicalcium phosphate or calcium from natural feeds. It is pointed out that the results of this survey of the biological availability of some of the common sources of calcium in livestock rations are not conclusive, but are indicative of relative availability. The procedures employed in this study make possible means for more intensive studies of factors causing the individual variations in the observed utilization values.

Summary

Radioisotope procedures were employed for the measurement of endogenous calcium in cattle and for the subsequent calculation of net absorption.

Results of availability studies from 109 individual balance studies with young and mature steers, pair-fed a basal ration to which calcium was supplied from 15 organic and inorganic sources indicated that true digestibility was greater in young than mature steers, and that this difference due to age was greater than that due to calcium source itself. Calcium from inorganic sources appeared to be utilized slightly more efficiently by cattle than that from alfalfa, lespedeza or orchard grass hay, but in consideration of the limited trials these differences were not statistically significant.

The application of the procedures described should be useful for the estimation of endogenous losses and the subsequent calculation of biological availability of calcium and various other minerals for cattle, sheep and swine under various experimental conditions.

Literature Cited

Armstrong, R. H. and Brynmor Thomas. 1952. The availability of calcium in three legumes of grassland. J. Agr. Sci. 42:455.

Comar, C. L., Sam L. Hansard, S. L. Hood, M. P. Plumlee and B. F. Barrentine. 1951. Use of Ca-45 in biological studies. Nucleonics 9:19.

Drake, T. G., S. H. Jackson, F. F. Tisdall and W. M. Johnstone. 1949. The biological availability of calcium in bone. J. Nutr. 37:369.

Ellenberger, H. B., J. A. Newland and C. H. Jones. 1931, 1932, 1950. Calcium and phosphorus requirements of dairy cows. Vt. Agr. Exp. Sta. Bul. 331:342:558.

Forbes, E. B., R. B. French and I. V. Letonoff. 1929. The mineral metabolism of the beef steer. J. Nutr. 1:201.

Hansard, Sam L. 1956. Tracer methods in mineral nutrition research. Michigan Isotope Conference. US AEC-TID (In press).

Hansard, Sam L., C. L. Comar and M. P. Plumlee. 1951. Radioisotope procedure with farm animals. Nucleonics 9:13; 38.

Hansard, Sam L., C. L. Comar and M. P. Plumlee. 1954. The effects of age upon calcium utilization and maintenance requirements in the bovine. J. Animal Sci. 13:25.

Hart, E. B., H. Steenbock, C. A. Hoppert and G. C. Humphrey. 1922. Dietary factors influencing calcium assimilation. J. Biol. Chem. 53:21.

Hayden, C. C., C. F. Monroe and C. H. Crawford. 1930. Dicalcium phosphate as a mineral supplement for dairy cattle. Ohio Agr. Exp. Sta. Bul. 455.

Lantow, J. L. 1933. The assimilation of calcium and phosphorus from different compounds and their effect on range cattle. New Mexico Agr. Exp. Sta. Bul. 214.

Lindsey, J. B. and J. G. Archibald. 1925. The value of calcium phosphate as a supplement to the ration of dairy cows. J. Agr. Res. 31:771.

Moxon, A. L., T. M. Paulsen and L. E. Johnson. 1947. Blood calcium and phosphorus level in range cattle. J. Animal Sci. 6:489.

Miller, R. C. 1926. A study of the calcium and phosphorus balance with dairy cows. J. Dairy Sci. 9:78.

Mitchell, H. H. and E. G. Curzon. 1939. The requirements of calcium and its significance. Acta. Scient. Ind. 771. Nutr. XVIII.

Sur, B. K. and V. Surbrahmanyan. 1952. Availability of calcium in lucerne and its value in nutrition. Indian J. Med. Res. 40:481.

Turner, W. A., T. S. Hardin and A. M. Hartman. 1927. The relative assimilation by dairy cows of clover and alfalfa hays and of rations of different calcium and phosphorus content. J. Agr. Res. 35:625.

Visek, W. J., R. A. Monroe, E. W. Swanson and C. L. Comar. 1953. Determination of endogenous fecal calcium in cattle by a simple isotope dilution method. J. Nutr. 50:23.

Weber, A. D., C. W. McCampbell, J. S. Hughes and W. J. Peterson. 1940. Calcium in the nutrition of the fattening calf. Kansas Agr. Exp. Sta. Tech. Bul. 51.

AVAILABILITY OF PHOSPHORUS IN MINERAL SUPPLEMENTS FOR BEEF CATTLE

T. A. Long,[1] A. D. Tillman, A. B. Nelson, Willis D. Gallup and Bill Davis

Oklahoma Agricultural Experiment Station [2]

THE availability of phosphorus contained in different mineral supplements has been studied extensively with chicks (Bird *et al.*, 1945; Gillis *et al.*, 1948; Grau and Zweigart, 1953; Miller and Joukowsky, 1953; Gillis *et al.*, 1954) and with swine (Gobble *et al.*, 1956; Chapman *et al.*, 1955). Work of this nature with cattle includes a report by Long *et al.* (1956). The purpose of the present study was (1) to compare the effects of different levels of a supplement in which the phosphorus is highly available upon certain criteria used as measures of phosphorus nutrition, and (2) to evaluate several mineral supplements as sources of phosphorus for beef cattle.

Experimental Procedure

Trial 1. Nine grade Hereford steers weighing about 390 lb. were individually fed a low-phosphorus (0.09%) basal ration for a preliminary period of 66 days. They were then divided on the basis of body weight and plasma inorganic phosphorus levels into three groups of three each. The animals in group 1 were continued on the basal ration; those in groups 2 and 3 were fed the basal ration plus 0.05% and 0.10% supplemental phosphorus, respectively. The supplemental phosphorus was added as NaH_2PO_4. Calcium carbonate was used to adjust calcium:phosphorus ratios of the rations to 4:1. The composition of the basal ration in percent was cottonseed hulls, 36.1; dried beet pulp, 27.2; dehydrated alfalfa meal, 9.1; corn gluten meal, 9.1; urea, 0.1; cerelose, 18; vitamin A&D supplement, 0.2 and salt, 0.2.

The animals were kept indoors in three large pens equipped with stanchions, feeders, and waterers. They were stanchioned twice daily and fed all the ration they would consume within one hour. They were weighed at 14-day intervals after 12-hour shrink periods without feed or water. Blood samples for the determination of plasma inorganic phosphorus were taken by jugular puncture at the time of weighing. Phosphorus determinations were made colorimetrically by a modification of the method of Fiske and Subbarow (1925). The data were analyzed statistically by analysis of variance (Snedecor, 1946) and multiple range test (Duncan, 1955). The experimental feeding period was of 104 days duration.

[1] Present address: Department of Animal Nutrition, Pennsylvania State University.
[2] Departments of Animal Husbandry and Agricultural Chemistry, Stillwater.

Trial 2. This trial was similar to trial 1 in purpose and general procedure. Nine grade Hereford steers weighing about 350 lb. were fed a low-phosphorus (0.07%) basal ration for 33 days, and then divided as in trial 1 into 3 groups. Group 1 was continued on the basal ration. Groups 2 and 3 were fed the basal ration plus NaH_2PO_4 in amounts to provide 0.04% and 0.08% supplemental phosphorus, respectively. The animals were fed these rations for a period of 99 days and handled as described in trial 1. The composition of the basal ration in percent was cottonseed hulls, 36.3; dried beet pulp, 27.2; dehydrated alfalfa meal, 9.1; corn gluten meal, 3.7; urea, 1.0; cerelose, 22.1, vitamin A&D supplement, 0.1; and salt, 0.5.

TABLE 1. AVERAGE RESPONSE OF STEERS TO INCREASED AMOUNTS OF SUPPLEMENTAL PHOSPHORUS[a]

	Trial 1			Trial 2		
Group No.	1	2	3	1	2	3
No. of steers	3	3	3	3	3	3
Feeding period, days	104	104	104	99	99	99
Supplemental phosphorus, %	0.0	0.05	0.10	0.0	0.04	0.08
Total phosphorus, %	0.09	0.14	0.19	0.07	0.11	0.15
Initial weight, lb.	413	411	410	353	358	365
Final weight, lb.	434	539	550	383	438	467
Gain, lb.	21	128	140	30	80	102
Initial feed intake,[b] lb.	8.7	8.7	8.7	6.7	6.7	7.3
Final feed intake,[b] lb.	4.7	10.0	10.0	5.4	9.7	12.3
Increase in feed intake, lb.	−4.0	1.3	1.3	−1.3	3.0	5.0
Average feed intake,[c] lb.	6.6	9.5	9.5	5.7	7.8	9.2
Initial plasma phosphorus, mg. %	3.9	3.8	3.6	4.2	4.3	5.0
Final plasma phosphorus, mg. %	2.9	5.4	6.1	2.8	4.2	5.8

[a] All steers were fed the basal rations for 66 days preceding trial 1 and 33 preceding trial 2.
[b] Feed intake on a daily basis over a two-week period.
[c] Feed intake on a daily basis for entire period.

Trial 3. This trial was a comparison of different phosphorus supplements fed at a level below that recommended for maximum gain. Thirty-one grade Hereford heifers weighing approximately 450 lb. were group-fed for a preliminary period of 33 days the same low-phosphorus (0.07%) basal ration as used in trial 2. The animals were then allotted at random into three groups of eight and one group of seven (steamed bone meal). One group continued to receive the basal ration; the other groups received the basal plus 0.08% supplemental phosphorus supplied by one of the three supplements, domestic steamed bone meal, Curacao Island phosphate and feeding grade dicalcium phosphate. All lots were group-fed from self-feeders during the 99-day experimental period. The feeders were cleaned at monthly intervals, and feed consumption calculated. The animals were weighed and bled as described in trial 1.

Results and Discussion

Trials 1 and 2. Feed intake and gains of the steers are shown in table 1. In trial 1, average feed intake of the three groups of steers at the beginning of the experimental period was 8.7 lb. per day. Feed intake of the steers on the basal ration (group 1) slowly decreased to 4.7 lb. per day at the end of the period, and the steers made an average total gain of only 21 lb. Steers on the supplemented rations, 0.14 and 0.19% phosphorus, progressively increased their feed intake to 10 lb. per day and made gains of 128 and 140 lb., respectively. Differences in gain between both supplemented groups (groups 2 and 3) and the basal group were statistically significant, ($P<.05$) as determined by multiple range test (Duncan, 1955); the difference in

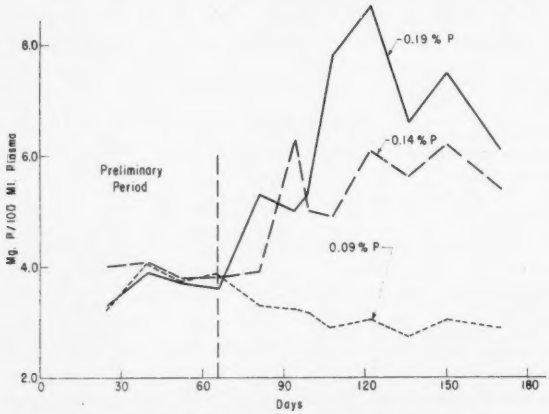

Figure 1. Inorganic plasma phosphorus values of steers fed three different levels of total phosphorus (Trial 1).

gain between groups 2 and 3 was not. The difference between the supplemented groups in final plasma phosphorus values (figure 1) likewise failed to show significance although these values were significantly higher ($P<.05$) for both groups than for the basal group. Initially the three groups had values of 3.9, 3.8 and 3.6 mg.; final values were 2.9, 5.4, and 6.1 for groups 1, 2 and 3, respectively. Thus from trial 1, it might be concluded that the total phosphorus content of rations to be used in the evaluation of phosphorus supplements, based upon their effect on feed intake, gain, and plasma phosphorus values, should not exceed 0.14%; unless, of course, the supplement makes up a large proportion of the total phosphorus and is relatively insoluble.

In trial 2, feed intake of the steers (group 1) on the basal ration, 0.07% phosphorus, decreased from 6.7 lb. to 5.4 lb., and the steers gained an average of 30 lb. Those on the 0.11% phosphorus ration (group 2) increased their feed intake from 6.7 lb. to 9.7 lb. and gained 80 lb.; and

those on the 0.15% ration (group 3) increased their intake from 7.3 lb. to 12.3 lb. and gained 102 lb. The weight gain of the steers in groups 2 and 3 was significantly different ($P<.05$) from the gain of those fed the basal ration (Duncan, 1955). Also, the feed consumption of the steers in groups 2 and 3 was significantly greater ($P<.01$) than the feed consumption of the group 1 steers. Neither the difference in weight gain or increase in feed consumption between groups 2 and 3 was statistically significant. Steers on the basal ration required 88% more feed per unit of gain and ate 39% less feed than those on the 0.11% ration. It appears, therefore, in agreement with Kleiber *et al.* (1936), that feed efficiency of steers on low phosphorus rations is affected even more markedly than feed intake. Phos-

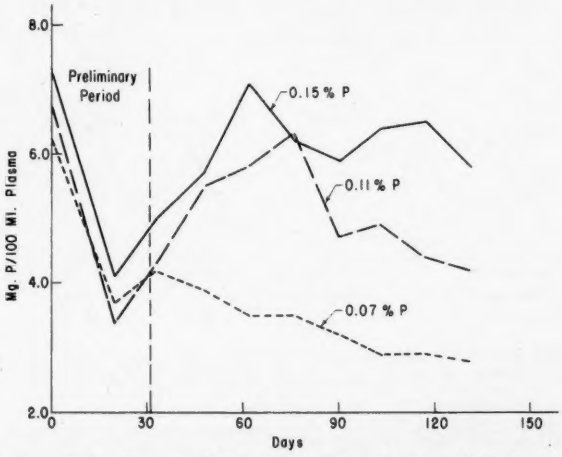

Figure 2. Inorganic plasma phosphorus values of steers fed three different levels of total phosphorus (Trial 2).

phorus deficiency symptoms, outwardly manifested by the steers fed the basal ration containing 0.07% phosphorus, were less evident or entirely absent in those fed the supplemented rations.

Average plasma phosphorus values which, at the beginning of the experimental period, were 4.2, 4.3 and 5.0 mg. for groups 1, 2 and 3, respectively, were 2.8, 4.2 and 5.8 at the end (figure 2). Analysis of the data (Duncan, 1955) showed that final values for group 3 (0.15% phosphorus) were not statistically different from those of group 2 (0.11% phosphorus), although higher ($P<.05$) for both these groups than for group 1.

The results of both trials suggest that at low levels of phosphorus intake, plasma phosphorus values are especially sensitive to change in intake, and it logically follows that they should be indicative of phosphorus availability in supplements fed at relatively low levels. For example, in trial 2, 0.04% soluble phosphorus added to a basal ration containing 0.07% feed

phosphorus increased the plasma level from 4.3 to 5.5 within 15 days. During the same interval, plasma phosphorus in steers not fed a supplement decreased from 4.2 to 3.9. Differences in feed consumption and weight as related to phosphorus intake, however, were not detectable until after the fourth or fifth week.

The time required to effect changes in plasma level is determined largely by the amount of soluble phosphorus ingested, as demonstrated in the experiments of Davis *et al.* (1956). For example, in that work plasma phosphorus values in steers given NaH_2PO_4 in a single dose in amounts equivalent to 7.5 gm. per 100 lb. body weight nearly doubled within 4

TABLE 2. AVERAGE RESPONSE OF HEIFERS TO DIFFERENT PHOSPHORUS SUPPLEMENTS [a] (Feeding period, 99 days)

Group No.	1	2	3	4
No. of heifers	8	7	8	8
Supplement	None	Bone meal	Curacao Is. phosphate	Dicalcium phosphate
Ration phosphorus, %	0.07	0.15	0.15	0.15
Initial weight, lb.	477	467	444	443
Final weight, lb.	491	584	579	561
Gain, lb.	14	117	135	118
Initial feed intake,[b] lb.	13.2	15.1	13.6	13.5
Final feed intake,[b] lb.	8.2	15.8	17.4	15.9
Increase in feed intake, lb.	−5.0	0.7	3.8	2.4
Av. feed intake,[c] lb.	11.1	16.2	16.1	15.6
Initial plasma phosphorus, mg. %	3.3	2.8	3.3	3.5
Final plasma phosphorus, mg. %	2.4	4.5	4.3	3.9

[a] All heifers were fed the basal ration for 33 days preceding the trial.
[b] Feed intake on a daily basis over 20- to 29-day periods.
[c] Feed intake on a daily basis over entire period.

hours. In the present experiments, maximum phosphorus intake from both feed and supplement was only 1.8 gm. per 100 lb. body weight. Black *et al.* (1943) found that incipient aphosphorosis in cattle could be diagnosed from plasma phosphorus values before physical symptoms were apparent. His observations have been duplicated in numerous studies. Nevertheless, change in plasma phosphorus related to phosphorus intake but affected by environmental factors and management of animals is also a common observation (Palmer *et al.*, 1930); consequently, as measures of change in nutritional status, such observations are of greatest value when made under controlled environmental conditions.

Apparently, the amount of phosphorus provided in the high-phosphorus ration of trial 1, 0.19%, approached minimum requirements, whereas, the lower amounts of phosphorus in the rations of trial 2, 0.11% and 0.15%, were within the critical range desired for studying the response of animals to change in intake and availability of phosphorus.

Trial 3. Table 2 shows the feed intake and gain in weight of heifers of

group 1 fed the basal ration (0.07% phosphorus) and those of groups 2, 3 and 4 fed the basal supplemented with 0.08% phosphorus derived from steamed bone meal, Curacao Island phosphate and dicalcium, respectively. Heifers in group 1 declined in feed intake from 13.2 lb. per day at the beginning of the trial to 8.2 lb. at the end, and they gained only 14 lb. Their plasma phosphorus values (figure 3) dropped from 3.3 to 2.4 mg. %.

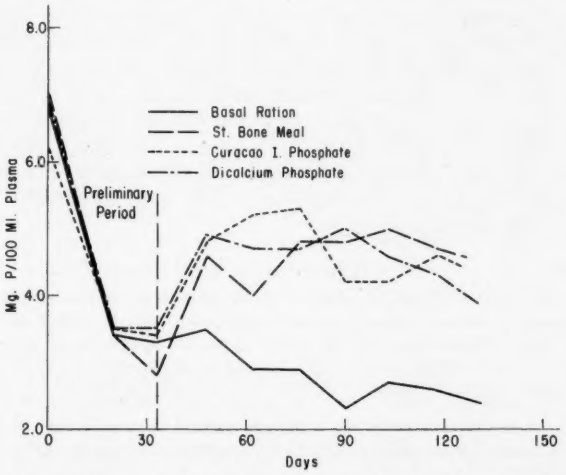

Figure 3. Inorganic plasma phosphorus values of heifers fed different sources of supplemental phosphorus (Trial 3).

Heifers in groups 2, 3 and 4 increased in feed intake and gained 117, 135 and 118 lb., respectively. Their plasma phosphorus values, which increased early in the trial, remained well above those of the basal group throughout and were 4.5, 4.3 and 3.9 mg. at the end. Their feed requirement per pound of gain was 10.8, 10.6 and 11.8 lb., respectively, as compared with 70.8 lb. for the basal group. Differences between the supplemented groups in these different measures of response to phosphorus source were not statistically significant at the 5% level of probability, as determined by multiple range test (Duncan, 1955). Although weight gains and feed efficiency favored slightly the group fed Curacao Island phosphate, the fact that other criteria, including plasma phosphorus level and general condition, did not, lends support to the conclusion to be drawn from the statistical analysis that the three supplements were of equal value in supplying phosphorus in the rations.

Summary

Two feeding trials were conducted with grade Hereford steers to compare the effects of different percentages of supplemental phosphorus on feed

consumption, weight gain and inorganic phosphorus in the plasma as measures of phosphorus nutrition. The steers were individually fed low-phosphorus (0.07 and 0.09%) rations alone and supplemented with NaH_2PO_4 in amounts to provide from 0.11 to 0.19% total phosphorus.

Feed intake, weight gain and plasma phosphorus increased with increased amounts of supplemental phosphorus in the ration over the range of 0.07, 0.11, 0.15 and 0.19% total phosphorus. Plasma phosphorus was especially sensitive to change in phosphorus intake but showed periodic variation.

Steamed bone meal, Curacao Island phosphate and dicalcium phosphate were compared as sources of phosphorus with heifers fed rations containing a total of 0.15% phosphorus. Heifers on the 0.07% basal ration declined in feed intake, made small gains, and their plasma phosphorus decreased. Those on the supplemented rations were normal according to these criteria, and any difference between them related to phosphorus source was not statistically significant. The results indicated equal availability of phosphorus in the three supplements.

Literature Cited

Ammerman, C. B., A. L. Neumann, R. M. Forbes and H. W. Norton. 1954. Utilization of phosphorus from various inorganic sources by steers. J. Animal Sci. 13:974.

Bird, H. R., J. P. Mattingly, H. W. Titus, J. C. Hammond, W. L. Kellogg, T. B. Clark, C. E. Weakley, Jr. and A. H. Van Landingham. 1945. Nutritive evaluation of defluorinated phosphates and other phosphorus supplements. J. Assn. Offic. Agr. Chem. 28:118.

Chapman, H. L., Jr., J. Kastelic, G. C. Ashton and D. V. Catron. 1955. A comparison of phosphorus from different sources for growing and finishing swine. J. Animal Sci. 14:1073.

Davis, Bill, T. A. Long, A. B. Nelson and Willis D. Gallup. 1956. Response of cattle to high phosphorus intake. Tetrasectional meet. Amer. Chem. Soc., Ponca City, Oklahoma.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 2:1.

Fiske, C. H. and Y. Subbarow. 1925. Colorimetric determinations of phosphorus. J. Biol. Chem. 66:375.

Gillis, M. G., L. C. Norris and G. F. Heuser. 1948. The utilization by the chick of inorganic phosphates. J. Nutr. 52:115.

Gillis, M. G., L. C. Norris and G. F. Heuser. 1948. The utilization by the chick of phosphorus from different sources. Poul. Sci. 28:283.

Gobble, J. L., R. C. Miller, G. W. Sherritt, and H. W. Dunne. 1956. Soft phosphate with colloidal clay as a source of phosphorus for grazing and fattening pigs. Penn. Agr. Exp. Sta. Bul. 609.

Grau, C. R. and P. A. Zweigart. 1953. Phosphatic clay as a phosphorus source for chicks. Poul. Sci. 32:500.

Kleiber, Max, H. Goss and H. R. Guilbert. 1936. Phosphorus deficiency metabolism and food utilization in beef heifers. J. Nutr. 12:121.

Long, T. A., A. D. Tillman, A. B. Nelson, Bill Davis and Willis D. Gallup. 1956. Dicalcium phosphate and soft phosphate with colloidal clay as sources of phosphorus for beef heifers. J. Animal Sci. 15:1112.

Miller, M. W. and V. V. Joukovsky. 1953. Availability of phosphorus from various phosphate material for chicks. Poul. Sci. 32:78.

Palmer, L. A., W. S. Cunningham and C. H. Eckles. 1930. Normal variations in the inorganic phosphorus of the blood of dairy cattle. J. Dairy Sci. 13:174.

Snedecor, G. W. 1946. Statistical Methods. Iowa State College Press, Ames.

EVALUATION BY LAMB FEEDING TESTS OF ALFALFA HAY GROWN ON A LOW-SULFUR SOIL [1]

V. V. Rendig and W. C. Weir
University of California, Davis [2]

An earlier report described the effect of sulfur fertilization on the chemical composition of alfalfa grown on a soil low in sulfur (Rendig, 1956). This paper describes various lamb feeding tests to determine the effect of fertilization on nutritive value.

Sulfur in animal nutrition is primarily involved with the sulfur-containing amino acids, cystine and methionine. It was indicated by the results of Rose and associates (1938) and confirmed by Tarver and Schmidt (1939) that the sulfur of methionine can be used to synthesize cystine in the animal body. In ruminants, at least, inorganic sources can supply some of the need for this element. Block *et al.* (1950, 1951) found that radioactive S^{35} fed as sodium sulfate was recovered in the protein fraction of goats milk and in both cystine and methionine in the rumen contents of the ewe.

Studies of the feeding value of alfalfa protein have indicated a relative deficiency of the sulfur-containing amino acids. Haag (1931) reported that cystine was the limiting factor for rats. More recently, Block and Mitchell (1946–47) have evaluated the "biological value" of alfalfa protein and found it to be deficient in isoleucine and methionine.

Experimental Methods and Results

Inasmuch as this study involved a series of separate tests the experimental plan and results obtained will be described for each in chronological order. Determinations of total nitrogen and sulfur were carried out in connection with every test. The Kjeldahl-Wilfarth-Gunning method (A.O.A.C., 1950) was used for determining total nitrogen. A method involving ignition in the presence of magnesium nitrate (A.O.A.C., 1950) was used for determining total sulfur. The method of Ellis *et al.* (1946) was employed for lignin.

For the first test, in 1950, alfalfa hay was obtained from two fields in Merced County, California. One field of 10 acres was Delhi sand (young, wind-modified granitic alluvium) and had shown marked yield responses as a result of applying sulfur-containing fertilizers. The other of about 5 acres was Hanford silt loam (young, well-drained granitic alluvium) and no benefit in yield was shown by sulfur fertilization. Both fields had been

[1] This study was supported in part by a grant from the American Dehydrators Association, Kansas City, Missouri. Methionine was furnished by courtesy of the Dow Chemical Company, Midland, Michigan, and urea by courtesy of E. I. DuPont de Nemours and Company, Inc. The assistance of the Agricultural Extension Service in obtaining hay for the various tests is gratefully acknowledged.

[2] Departments of Soils and Plant Nutrition and of Animal Husbandry.

seeded to alfalfa in 1949 and were flood irrigated. Single superphosphate (approx. 18% P_2O_5, 11.5% S) was top-dressed to alternate irrigation checks in each field at the rate of 400 lb. per acre in February, 1950. Although alfalfa grown on soils similar to the Delhi sand had shown by chemical analysis a greater increase in sulfur than in phosphorus content, it was deemed advisable to apply single superphosphate to insure adequate phosphorus.

To test the nutritive value of the alfalfa, it was brought to the Experiment Station at Davis and fed to lambs. The trial was begun October 28, 1950, and ended March 22, 1951, a total of 147 days. The lambs used were well-grown, Columbia-type ewe lambs from the University flock and averaged 99 lb. at the beginning of the trial. They were divided on the basis of body weight into eight lots of four lambs each. All lots were made as uniform as possible. Duplicate lots received one of the following kinds of hay: (1) fertilized Hanford, (2) unfertilized Hanford, (3) fertilized Delhi, and (4) unfertilized Delhi. Since weed growth was most prevalent in the spring and early summer cuttings, hay for the test was not obtained until midseason. The third and fourth cuttings from the Delhi and one cutting from the Hanford soil were used.

The hay was chopped to reduce the possibility of the animals sorting the leaves from the stems. Sugar (cerelose) was added initially at the level of 1 part of sugar to 9 parts of hay. After 72 days of the trial, the sugar was increased to 2 parts for each 9 parts hay and was continued at that level. The desirability of using a supplemental energy source for such studies was indicated from the results of Matrone *et al.* (1949). The lambs were fed once a day. To reduce variability of intake, each lot was given the same quantity of feed determined by the lot that ate the least. The animals were weighed monthly and at intervening times when they were changed to different cuttings of the hay. Blood samples were taken at weighing time and the serum analyzed for inorganic sulfate by the method of Letonoff and Reinhold (1936). Results indicating the relationship of sulfur intake to serum sulfate level were published previously (Weir and Rendig, 1954). Whiting *et al.* (1954) using different analytical methods subsequently reported that rations containing less sulfur than those used by the authors did not result in a lower serum sulfate level.

The results of the first feeding trial are shown in table 1. The rate of gain of the lambs fed the unfertilized Delhi (low S) hay was consistently less than that of any other group. This gain was significantly less than the gain of either group on the Hanford (high S) hay but the difference between the Delhi hays—fertilized and unfertilized—was not significant. One of the lots fed the unfertilized Delhi hay was the lot which consistently limited the level fed all lots.

During the course of the feeding trial the serum inorganic sulfate of the lambs fed the Hanford hay increased; that of the lambs fed both the Delhi hays decreased. The decrease was more rapid in the lambs fed the

unfertilized Delhi hay. The final difference in blood sulfate level of the lambs fed the Hanford hay and the Delhi hay was highly significant. The results obtained from the blood serum analysis are consistent with those presented in an earlier publication (Weir and Rendig, 1954). Serum calcium and phosphate levels were also determined but the averages for each lot were essentially the same.

The following year a test was organized to determine whether the feeding value of low-sulfur alfalfa could be changed by supplementing the hay with methionine or urea. The purpose of the former was to furnish a source of organic sulfur, the latter to change the nitrogen/sulfur ratio. It

TABLE 1. COMPOSITION OF HAY GROWN IN 1950 AND RESULTS OF 147-DAY FEEDING TRIAL

Source of hay [a]	Total sulfur	Total nitrogen	N/S	Lignin	Av. daily feed	Av. daily gain	Final serum sulfate level
	%	%		%	lb.	lb.	mg./100 ml.
Hanford F	0.275	2.96	10.8	7.83	2.70	0.153	4.13
Hanford U	0.257	3.00	11.7	7.81	2.70	0.150	4.23
Delhi F (4)	0.143	2.09	14.6	10.15	2.66	0.117	2.69
Delhi F (3)	0.216	2.64	12.1	9.26			1.40
Delhi U (4)	0.125	2.11	16.8	11.33	2.64	0.084	0.51
Delhi U (3)	0.153	2.62	17.0	10.06			1.14
LSD (5% level)						0.038	0.064

[a] F = fertilized; U = unfertilized; numbers in parentheses refer to cutting.

has been reported that widening of this ratio above 15:1 may adversely affect the utilization of sulfur (Loosli, 1952).

The trial was carried out in essentially the same manner as in the previous year. Thirty-six Corriedale-type lambs averaging 69 lb. were divided on the basis of weight into six lots. Each lot was fed the unfertilized Delhi (low S) hay plus 18% sugar. In addition, two of the lots were fed 0.68% methionine and two others 0.42% urea. These supplements were mixed with the sugar in water and poured over the chopped hay the night before feeding.

The results of the second test are shown in table 2. The feed intake and average daily gains were much below those obtained in the previous test. The lambs fed the urea-supplemented hay consistently ate the least and thus limited the intake of all of the lots. The average daily gain of the lambs fed the hay plus methionine was higher than that for the other lots but only when compared with the urea-supplemented lots was the difference significant. The average serum sulfate level for lambs receiving methionine-

TABLE 2. EFFECT OF METHIONINE AND OF UREA SUPPLEMENTS ON THE AVERAGE DAILY GAIN OF LAMBS FED ALFALFA HAY GROWN ON A LOW-SULFUR SOIL (1951)

Supplement	Total S content of ration	N/S ratio of ration	Av. daily feed	Av. daily gain
	%		lb.	lb.
Sugar only	0.129	17.1	1.99	0.127
Sugar + 0.68%[a] methionine	0.275	8.1	1.98	0.141
Sugar + 0.42%[a] urea	0.129	18.7	1.96	0.101
LSD (5% level)				0.031

[a] Based on dry weight of hay.

supplemented hay was significantly greater than the level for those receiving either hay alone or urea-supplemented hay (figure 1).

The third test was set up to compare the digestibility of fertilized and unfertilized hay grown on a low-sulfur soil with that grown on a soil which did not produce a greater yield of alfalfa when sulfur fertilized than when unfertilized. The effect of adding methionine to the latter hay and to that grown without fertilization on a low-sulfur soil was also investigated. Both feces and urine samples were collected so that the amount of sulfur and nitrogen retained could be calculated.

The low-sulfur hay (Delhi) for this test was obtained from the same location as that used in previous tests. It was cut near the end of the season (fifth cutting) so was not as low in sulfur as would be the case for

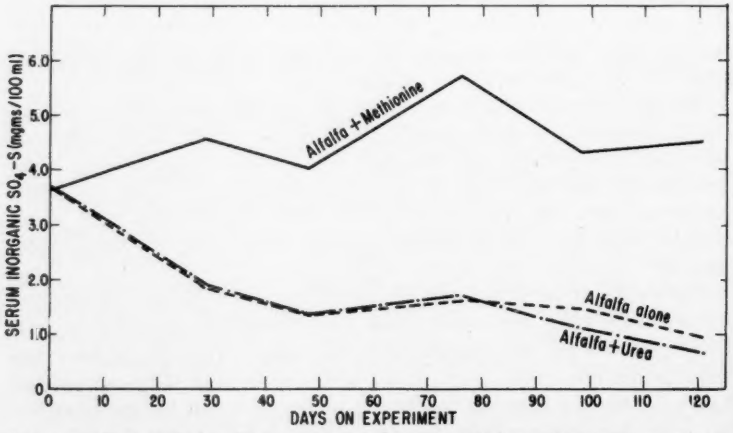

Figure 1. The variation in the serum inorganic sulfate sulfur level of lambs fed alfalfa (low S) alone and with supplements indicated.

hay grown in midseason (Rendig, 1956). The alfalfa used for comparison was obtained from a field near Davis, California, on which the soil is of the Yolo (young, from sedimentary rock alluvium) series. The hay at both locations was field-cured, ground rather finely, mixed with supplements and then pelleted. Sugar in an amount equal to 18% of the dry weight was added to all lots. Sufficient methionine was added to one lot of the unfertilized Delhi and one lot of the Yolo hay so that the total sulfur content was approximately the same as that contained in the fertilized Delhi hay (table 3).

TABLE 3. COMPOSITION, DIGESTION COEFFICIENTS AND TDN OF ALFALFA HAY-SUGAR PELLETS (1952)

Lot		Crude protein	Crude fiber	Ether extract	NFE	TDN		Lignin
						Conv.	Lofgreen	
		%	%	%	%			%
Yolo hay	Comp.	16.4	25.2	2.04	45.8	60.2	59.4	7.15
	Dig. coef.	73.0	41.9	49.8	75.9			
Yolo hay+meth.	Comp.	16.4	26.1	1.73	46.3	59.7	59.3	7.78
	Dig. coef.	73.8	38.9	30.4	73.7			
Delhi fertilized hay	Comp.	18.0	19.6	2.30	50.0	65.5	64.9	6.05
	Dig. coef.	75.3	45.6	48.0	78.8			
Delhi unfertilized hay	Comp.	15.4	23.2	1.84	51.2	61.5	61.8	7.43
	Dig. coef.	71.7	33.9	27.6	79.5			
Delhi unfert. hay+meth.	Comp.	16.5	23.2	1.73	48.8	61.4	61.2	7.4
	Dig. coef.	74.9	37.9	19.2	78.5			
LSD (5% level) of digestion coefficients		NS	9.1	12.7	NS	NS	NS	

The pellets were fed to wether lambs in digestion racks for a period of 14 days at a constant level calculated as 110% of maintenance. Distilled water was used throughout. Feces and urine samples were collected for the latter 7 days of the trial. The tests were carried out in duplicate.

The digestion coefficients obtained in the trial are shown in table 3. The digestibility of the crude fiber in the unfertilized Delhi was significantly less than that in the fertilized Delhi hay. The addition of methionine had no significant effect on crude fiber digestibility. The digestibility of the ether extract was also somewhat less in the unfertilized Delhi hay. Since this fraction only accounts for 2–3% of the total dry weight of the hay the coefficients varied widely for each lot as evidenced by the high LSD. The total digestible nutrients were determined by both the conventional method and the method of Lofgreen (1953). Good agreement was obtained

TABLE 4. SULFUR BALANCE DATA FOR LAMBS FED ALFALFA HAY-SUGAR PELLETS (1952)

Source of hay	Sulfur content of pellets	S intake/day			S retention/day	
		Total S	Meth. supp.	SO_4–S	Total S	SO_4–S[b]
	%	gm.	gm.	gm.	gm.	gm.
Yolo	0.245	2.79	—	1.18	+0.24	+0.03
Yolo	0.307[a]	3.45	0.69	1.18	+0.18	−0.62
Delhi fertilized	0.297	3.32	—	1.19	+0.51	−0.28
Delhi unfertilized	0.173	1.86	—	0.33	+0.42	−0.04
Delhi unfertilized	0.316[a]	3.90	1.77	0.48	+0.49	−1.34
LSD (5% level)					+0.23	

[a] Includes methionine added.
[b] Only urinary sulfate measured.

by the two methods but none of the differences between the values obtained for the different lots of pellets were significant.

The sulfur balance data are shown in table 4. The results indicate that sulfur retention is not related to sulfur intake at the levels used in this test. Significantly less sulfur was retained by lambs fed the Yolo hay than those fed the Delhi hay. No explanation for this difference can be offered at the present time. The addition of methionine had no significant effect on sulfur retention. The difference in urinary excretion of sulfate sulfur by lambs receiving the amino acid, compared to those not receiving it, equalled the amount of sulfur added. Presumably the methionine was metabolized. There was no significant difference between the lots in the amount of nitrogen retained.

The most recent alfalfa evaluation test was carried out with hay from another sulfur deficient area in Butte County, California. The soil in the field selected had been mapped as the Farwell series (from basic igneous alluvium). Gypsum at the rate of 400 lb. per acre was applied to alternate irrigation checks. Sufficient hay was obtained so that both growth perform-

TABLE 5. RESULTS OF LAMB FEEDING TRIAL WITH RATIONS PREPARED FROM HAY GROWN ON LOW-SULFUR FARWELL SOIL (1955)

Ration	Sulfur content[a]	Av. daily gain	Feed/100 lb. gain
	%	lb.	lb.
Fertilized hay	0.262	0.387±.013	929
Unfertilized hay	0.155	0.358±.071	994
Unfertilized hay + methionine	0.234	0.354±.063	1002
LSD (5% level)	0.020	NS	NS

[a] Means represent average of composite samples collected during course of trial.

ance and digestibility and retention measurements could be made. For the feeding test thirty lambs averaging about 69 lb. were divided into three lots so that the average weights and range in weights were about the same for each lot. They were fed alfalfa in pelleted form as follows: (1) fertilized, (2) unfertilized, (3) unfertilized plus sufficient methionine to bring the sulfur content to almost the same level as that of the fertilized hay. Unlike the previous trials no sugar was added to the hay. It had been found in tests carried out in connection with another study that good weight gains could be obtained with alfalfa pellets fed alone. Rather than controlled group feeding as in previous trials, improved facilities made it possible to feed the lambs individually on an *ad lib.* basis. The animals were weighed and blood samples taken at monthly intervals.

TABLE 6. SULFUR INTAKE AND SERUM SULFATE LEVEL (1955)

Ration	Initial serum sulfate	Feeding periods: June 11–July 10 Av. S intake	June 11–July 10 SO_4–S in serum	July 11–Aug. 11 Av. S intake	July 11–Aug. 11 SO_4–S in serum	Aug. 12–Sept. 12 Av. S intake	Aug. 12–Sept. 12 SO_4–S in serum
	mg./100 ml.	gm./day	mg./100 ml.	gm./day	mg./100 ml.	gm./day	mg./100 ml.
Fertilized hay	2.84	4.06	3.60	4.10	3.24	4.76	3.55
Unfertilized hay	2.58	2.30	1.29	2.27	1.67	2.79	2.06
Unfert. hay + methionine	2.62	3.40	3.36	3.59	2.91	4.17	3.87
LSD (1% level)	NS	0.43	1.17	0.33	0.86	0.30	1.06

The growth data obtained in the 1955 trial are shown in table 5. Neither the differences in weight gains or the feed per 100 lb. gain are significant when tested by the analyses of variance. As shown by standard errors, lambs fed the fertilized hay gained much more uniformly than those fed either of the other two lots of pellets.

Serum sulfate levels were lower in lambs fed the unfertilized hay than in those fed either of the other two lots of pellets (table 6). While the difference in average daily sulfur intake of the lambs fed the sulfur fertilized alfalfa and the unfertilized alfalfa plus methionine was highly significant no difference was found in the serum sulfate value. This suggests that the latter value is not proportional to sulfur intake at least at higher intake levels. Results obtained in this and in previous tests indicate that there is a "critical" level of intake required to maintain a "normal" serum sulfate value. This becomes more apparent when the average daily sulfur intake for the individual lambs for each monthly feeding period is plotted against the serum sulfate value at the end of the respective month. For all lambs receiving the unfertilized hay the average daily sulfur intake for each

of the three monthly feeding periods was less than 3.00 gm. For only three out of a total of 30 determinations were values found greater than 2.50 mg. SO_4–S/100 ml. For the other two lots only seven out of a total of 57 determinations were found to be less than 2.50 mg. SO_4–S/100 ml. The average daily sulfur intake of these 19 lambs for each monthly period was, with three exceptions, at least 3 gm. per day.

There was no significant difference in the average retention of total sulfur between lambs fed the three lots of pellets (table 7). The values were comparable to those found in earlier tests. The excretion of sulfate sulfur was consistently greater than the intake. As before, the difference in sulfate sulfur excretion between lambs fed methionine-supplemented alfalfa and those fed alfalfa alone was almost equal to the amount of sulfur con-

TABLE 7. SULFUR BALANCE DATA (1955)

Pellet lot	Total S content	S intake/day			S retention/day	
		Total S	Meth. supp.	SO_4–S	Total S	SO_4–S
	%	gm.	gm.	gm.	gm.	gm.
Fertilized hay	0.310	2.82		0.99	0.37	—0.31
Unfertilized hay	0.160	1.45		0.18	0.36	—0.14
Unfertilized hay & meth.	0.247	2.18	0.57	0.19	0.29	—0.77
LSD (5% level)					NS	.06

tained in the methionine. The sulfate sulfur content of the feces was fairly constant for all lambs. Warth and Krishnan (1935) had reported that this value was related to the blood sulfate content. Although the serum sulfate level of the lambs used for the digestion trial was not measured, lambs previously fed pellets from the unfertilized hay had comparatively low serum sulfate levels.

The digestibility of the pellets from alfalfa grown on the Farwell soil was tested by feeding to lambs and employing a 3 x 3 Latin Square experimental design. Composition of the pellets and data obtained are shown in table 8. Digestion coefficients of the fertilized hay were consistently greater than those obtained with unfertilized hay whether supplemented with methionine or not but the differences were not significant. The total digestible nutrients were determined both by the conventional method and the "digestible organic matter" method described by Lofgreen (1953). Agreement between the two methods was good, both indicating that the fertilized hay was higher in total digestible nutrients.

Discussion

This series of trials studying the feeding value of alfalfa grown on low-sulfur soils with and without sulfur fertilizers have shown a trend toward improved feeding value of the alfalfa grown with fertilizers. However, it

has not always been possible to obtain a clear-cut, significant improvement as measured by animal performance. Attempts to improve the feeding value of low-sulfur hay by the addition of methionine have been unsuccessful. Lofgreen *et al.* (1954) also found no consistent benefit in gains or wool growth of lambs fed methionine in addition to a low-protein ration in which urea furnished 40% of the nitrogen. It appears that there is no practical benefit from the addition of methionine to alfalfa hay with sulfur contents such as those tested in these trials.

Evaluation of hay by comparative methods such as used in this investigation involve certain complications. If the hay is compared with that grown in another location, even though climatically very similar, usually

TABLE 8. COMPOSITION AND DIGESTION COEFFICIENTS (1955)

Lot		Crude protein	Crude fiber	Ether extract	NFE	TDN		Lignin
						Conv.	Lofgreen	
		%	%	%	%			%
Fertilized hay	Comp.	20.9	23.9	1.47	44.0	60.6	61.35	10.0
	Dig. coef.	77.3	42.5	20.5	76.2			
Unfertilized hay	Comp.	18.2	27.9	1.13	43.4	55.2	55.81	10.2
	Dig. coef.	73.9	34.8	10.4	73.2			
Unfertilized hay methionine	Comp.	18.5	28.0	1.37	42.8	56.5	57.03	9.7
	Dig. coef.	75.5	39.1	13.7	72.7			
LSD (.05) of Digestion Coefficients		NS	NS	NS	NS	4.56	3.92	

differences in composition other than those of special interest for a particular study also occur. In the first study (1950), for example, there was not only a marked difference in total sulfur content but also in lignin content between hays from the two locations. Recent studies (Meyer and Lofgreen, 1956) have shown the close relationship of total digestible nutrient to lignin content. However, the lignin content does not explain all differences in feeding value between alfalfa hays varying in sulfur content as indicated by the results of the last trial. Here, while the lignin content did not change as a result of the fertilizer treatment, there was a difference in the content of total digestible nutrients.

Even in comparisons of fertilized and unfertilized hay from a single location, differences in more than a single element or constituent occur. The application of an element to a soil deficient in that element commonly results in multiple changes in plant composition. It has been noted that a correction of a soil-sulfur deficiency by fertilization sometimes results in an increase in nitrogen content in alfalfa. On the same field this may be observed in only one of several cuttings made during a season (Rendig,

1956). In no case was any evidence obtained in this investigation that such increases by themselves enhance the feeding value of alfalfa. This does not mean that such is always the case with alfalfa or that this applies to other hays or forages. It does indicate that the use of "Kjeldahl Nitrogen" as a single measurement for assessing differences in quality could be misleading.

It would appear that the efficiency of utilization of the sulfur in the unfertilized alfalfa is comparable to that of methionine. Albert *et al.* (1956) found that the optimum level of methionine addition to a low-sulfur basal ration (0.048% S) was $0.640 \pm 0.048\%$, which calculated as sulfur would be $0.138 \pm .014\%$. With sodium sulfate, however, an addition of 1.27 ± 0.44 ($0.285 \pm .099\%$ S) was required for optimum growth. In the studies reported here alfalfa containing 0.155% total sulfur resulted in a good rate of gain (table 5).

Summary

The feeding value of alfalfa grown on soils which did and did not respond to sulfur fertilizers has been studied utilizing lamb gains, digestion trials and nitrogen and sulfur balance trials. A rather consistent, though not always significant, trend in animal performance indicated that alfalfa grown on low-sulfur soils was improved by fertilization. The addition of methionine to alfalfa hay considered low in sulfur did not improve the feeding value of the hay.

The application of sulfur-containing fertilizers to soils low in sulfur markedly increased the sulfur content of the alfalfa grown. Other accompanying changes in composition appeared to be of greater effect on the feeding value than the changes in sulfur content. No one chemical fraction appears to be of unique importance in determining the nutritive value of the hay in these studies.

The previous finding that low sulfur intake of sheep is reflected in the blood serum inorganic sulfate level has been further verified.

Literature Cited

Albert, W. W., U. S. Garrigus, R. M. Forbes and H. W. Norton. 1956. The sulfur requirement of growing-fattening lambs in terms of methionine, sodium sulfate and elemental sulfur. J. Animal Sci. 15:559.

Association of Official Agricultural Chemists. 1950. Official methods of analysis. 7th Ed. Washington, D. C.

Block, R. J. and H. H. Mitchell. 1946–47. The correlation of the amino acid composition of proteins with their nutritive value. Nutr. Abs. Revs. 16:249.

Block, R. J. and J. A. Stekol. 1950. Synthesis of sulfur amino acids from inorganic sulfate by ruminants. I. Proc. Soc. Exp. Biol. Med. 73:391.

Block, R. J., J. A. Stekol and J. K. Loosli. 1951. Synthesis of sulfur amino acids from inorganic sulfate by ruminants. II. Arch. Biochem. Biophys. 33:353.

Ellis, G. H., G. Matrone and L. A. Maynard. 1946. A 72 percent H_2SO_4 method for the determination of lignin and its use in animal nutrition studies. J. Animal Sci. 5:285.

Haag, J. R. 1931. The physiological effect of rations restricted principally or solely to the alfalfa plant. II. J. Nutr. 4:363.

Letonoff, T. V. and J. G. Reinhold. 1936. A colorimetric method for the determination of inorganic sulfate in serum and urine. J. Biol. Chem. 114:147.
Lofgreen, G. P. 1953. The estimation of total digestible nutrients from digestible organic matter. J. Animal Sci. 12:359.
Lofgreen, G. P., W. C. Weir and J. F. Wilson. 1954. Unpublished data. California Agricultural Experiment Station.
Loosli, J. K. 1952. Meeting the sulfur requirements of ruminants. Feed Age 2:44.
Matrone, G., R. L. Lovvorn, W. J. Peterson, F. H. Smith and J. A. Weybrew. 1949. Studies of the effect of phosphate fertilization on the composition and nutritive value of certain forages for sheep. J. Animal Sci. 8:41.
Meyer, J. H. and G. P. Lofgreen. 1956. The estimation of the total digestible nutrients in alfalfa from its lignin and crude fiber content. J. Animal Sci. 15:543.
Rendig, V. V. 1956. Sulfur and nitrogen composition of fertilized and unfertilized alfalfa grown on a sulfur-deficient soil. Soil Sci. Soc. Amer. Proc. 20:237.
Rose, W. C. 1938. The nutritive significance of the amino acids. Physiol. Rev. 18:109.
Tarver, H. and C. L. A. Schmidt. 1939. The conversion of methionine to cystine. J. Biol. Chem. 130:67.
Warth, F. J. and T. S. Krishnan. 1935. Sulfur and sulfate balance experiments with sheep. Indian J. Vet. Sci. An. Hus. 5:319.
Weir, W. C. and V. V. Rendig. 1954. Serum inorganic sulfate sulfur as a measure of the sulfur intake of sheep. J. Nutr. 54:87.
Whiting, F., S. B. Slen, L. M. Bezeau and R. D. Clark. 1954. The sulphur requirement of mature range ewes. Canadian J. Agr. Sci. 34:261.

THE EFFECT OF NITROGEN FERTILIZATION ON THE NUTRITIVE VALUE OF ORCHARDGRASS HAY [1]

B. R. POULTON,[2] G. J. MACDONALD [3] AND G. W. VANDER NOOT
New Jersey Agricultural Experiment Station

THE difficulties encountered in trying to maintain stands of alfalfa on certain soil types, particularly in the Northeast, has stimulated increasing interest in the production of better-quality grass hays. As feed for livestock, legume hays have been preferred over grass hays, principally because of their greater protein content.

Salisbury and Morrison (1938) demonstrated that the crude protein content of timothy hay could be increased to 9.38% by applying 250 lb. of calcium cyanamid per acre. In subsequent feeding trials this timothy hay compared favorably with alfalfa hay when daily milk production was used as a measure of nutritive worth. Burton and DeVane (1952) reported that the application of 0, 50, 100, 200, and 400 lb. of nitrogen to Coastal Bermuda grass resulted in hay yields of 2,100, 4,212, 7,448, 10,834 and 16,339 lb., per acre respectively on a 16% moisture basis. Marked increases in protein content of the grass was observed, due to the nitrogen application. At the highest level (400 lb. of nitrogen per acre), these authors reported an average crude protein content in the hay of 13.03%.

Wagner (1954) reported that the application of 240 lb. of nitrogen per acre on orchardgrass, yielded a forage that contained 18.51% crude protein on a dry-matter basis. Similar results have been reported by Ramage (1956) who found that over a 3-year period orchardgrass fertilized with 400 lb. of nitrogen per acre yielded 8,458 lb. of dry matter annually. This forage had an average crude protein content of 19.84% on a dry-matter basis.

These reports and others indicate that timely use of nitrogen on grasses can result in a forage yield with crude protein content comparable to that of legumes. It has been generally assumed that the percentage of true protein in the crude protein is less in these highly fertilized grass hays than in legumes. Greenhill, (1936) and Ferguson and Terry, (1954) reported that the relative non-protein nitrogen content of grasses increases in response to nitrogen fertilization.

This investigation sought to determine the digestibility of the crude protein, ether extract, crude fiber, and energy components of orchardgrass hay grown under varying levels of nitrogen fertilization, and to compare these data with the digestibility of alfalfa hay grown with conventional fer-

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Departments of Animal and Dairy Husbandry, New Brunswick. This study was partially financed by Regional Project NE-24.

[2] Present address, Department of Animal Industry, University of Maine, Orono, Maine.
[3] Research Assistant in Animal Husbandry.

tilizer applications. Also the TDN and digestible energy systems were compared as methods of evaluating the energy content of these hays.

Experimental Procedure

Four yearling wether sheep of uniform size and body weight were used in this study. Metabolism crates were constructed as described by Bratzler (1951). Each sheep was randomly assigned to one of the following rations: Ration A, Alfalfa hay, second cutting (cut July 7th), harvested at about the half-bloom stage of maturity, which served as a comparison standard for the other three rations. Ration 0–1, Orchardgrass hay, second cutting (cut July 7th), grown with 100 lb. of nitrogen per acre. Ration 0–2, Orchardgrass hay, second cutting (cut July 7th), grown with 200 lb. of nitrogen per acre. Ration 0–4, Orchardgrass hay, second cutting (cut July 7th), grown with 400 lb. of nitrogen per acre. All lots of orchardgrass received a basic fertilizer application of 100 lb. per acre of both potash and phosphoric acid.

All orchardgrass fields were cut three times a year during the 3 years that yield data were collected. The hays fed were the second cutting crop during the final year. The dry matter yield for this cutting from the 100, 200 and 400 lb. of nitrogen plots were 3192, 2994, and 3116 lb. of dry matter per acre, respectively. Complete yield data for the first two years of harvest were previously reported by Ramage *et al.* (1955).

The digestion trial was divided into a 10-day preliminary and a 10-day collection period. One of the rations was fed to each sheep during each period in a conventional latin-square design. The hays were fed in nearly equal amounts and the amounts refused were kept to a minimum. Feed and fecal samples were taken daily during the collection period. Protein, ether extract, and fiber analyses were carried out according to the procedure outlined by the A.O.A.C. Energy determinations were made in an Emmerson adiabatic-type fuel calorimeter.

Results and Discussion

Chemical analyses of the hays are presented in table 1. The protein content of the alfalfa hay was approximately equal to the level found in orchardgrass hay grown with 400 lb. of nitrogen per acre but was 0.67% and 4.30% higher than the orchardgrass hay grown with 200 and 100 lb. of nitrogen, respectively. Crude fiber, ash, and nitrogen-free extract content of all hays were approximately the same. The orchardgrass hays were 2% higher in ether extract content than the alfalfa hay. This difference was reflected in the higher gross energy values for the orchardgrass hays.

The digestion coefficients for the hays are presented in table 2. The digestibility of the protein in the orchardgrass hays grown with 200 and 400 lb. of nitrogen per acre was significantly ($P<0.05$) lower than the digestibility of the protein in alfalfa hay. Protein digestibility in the

TABLE 1. CHEMICAL ANALYSIS OF DRY MATTER IN HAYS FED

Hay fed	Crude protein	Crude fiber	Ether extract	Nitrogen-free extract	Ash	Gross energy
	%	%	%	%	%	cal./gm.
Alfalfa	19.19	34.19	1.87	38.72	7.03	4,575
Orchardgrass						
100 lb. nitrogen/A	13.89	35.77	4.00	38.24	8.10	4,633
200 lb. nitrogen/A	17.52	35.32	4.14	35.65	7.37	4,680
400 lb. nitrogen/A	18.02	35.59	3.98	35.54	6.87	4,679

orchardgrass hay grown with 100 lb. of nitrogen showed a highly significant ($P<0.01$) decrease when compared to the alfalfa hay.

The orchardgrass hays fed in this study were far superior to alfalfa in regard to the digestibility of crude fiber. Regardless of the amount of nitrogen applied all the orchardgrass hays had a higher digestible crude fiber content than did the alfalfa hay. A statistical analysis revealed that in each case this increase in fiber digestibility was highly significant ($P<0.01$). This superior digestibility of crude fiber in orchardgrass when compared to alfalfa was also reported by Swift *et al.* (1950). In our study the orchardgrass was cut at an earlier stage of growth than was the alfalfa and this undoubtedly contributed somewhat to the greater digestibility of the fiber content of orchardgrass. This cannot, however, be the only factor responsible for the large difference observed. Woodman *et al.* (1933) suggested that the crude fiber portion of alfalfa hay is normally more highly lignified than that of orchardgrass hay. Armstrong *et al.* (1950) reported lignin concentrations in the crude fiber portion of alfalfa ranging from 14 to 18% of the ash-free dry matter whereas the concentrations observed in orchardgrass ranged only from 5 to 6%. The total amount of lignin present in the two crops was very similar; however, a much smaller percentage of the lignin present in orchardgrass was recovered in the crude fiber analysis.

The differences observed in the digestibility of the energy, ether extract,

TABLE 2. DIGESTION COEFFICIENTS AND DIGESTIBLE ENERGY AND TDN CONTENT OF THE HAYS FED

Hay fed	Crude protein	Crude fiber	Ether extract	Nitrogen-free extract	Energy	Digestible energy	Total digestible nutrients
	%	%	%	%	%	cal./gm.	%
Alfalfa	78.3	39.4	12.6	77.5	59.6	2,726	58.2
Orchardgrass							
100 lb. nitrogen/A	67.4	73.2	24.5	70.0	64.1	2,970	64.5
200 lb. nitrogen/A	73.8	69.0	34.1	68.8	63.6	2,975	65.0
400 lb. nitrogen/A	73.8	70.0	33.4	66.7	63.4	2,969	64.1

and nitrogen-free extract components of these hays were not significant. It is interesting to note, however, that the digestibility of the nitrogen-free extract portion of the alfalfa hay was higher than for the orchardgrass hays. This may be due to a greater amount of lignin being present in the nitrogen-free extract component of the orchardgrass hays.

The total digestible nutrient and digestible energy values of the four hays fed are presented in table 2. Whether evaluated on their TDN content or their digestible energy content the results for these four hays are similar. The three orchardgrass hays were approximately equal in their TDN and digestible energy content and they were all superior to the alfalfa hay. An evaluation of the energy content of these four hays on a TDN basis shows that the orchardgrass hays contained approximately 10% more energy than the alfalfa hay. A similar evaluation on a digestible energy basis showed a 9% greater energy content for the orchardgrass hays. It is apparent from this comparison that in this study digestible energy was a satisfactory substitute for TDN in evaluating the energy content of hay.

Summary

A digestion trial was carried out using four yearling wether sheep. A 4 x 4 latin-square design was followed. Orchardgrass hays, that had been grown with 100, 200, and 400 lb. of nitrogen per acre were compared with a standard alfalfa hay. Digestible energy was also determined.

The apparent digestibility of the crude protein in the orchardgrass hays was significantly lower than that of alfalfa hay. Crude fiber digestibility for the orchardgrass hays was much higher than for the alfalfa. This difference was highly significant. No significant variation in the digestibility of the energy, ether extract, and nitrogen-free extract components was observed.

The TDN and digestible energy values of the three orchardgrass hays were considerably higher than the values found for the alfalfa hay.

Literature Cited

Armstrong, D. G., H. Cook and B. Thomas. 1950. The lignin and cellulose contents of certain grassland species at different stages of growth. J. Agr. Sci. 40:93.

Bratzler, J. W. 1951. A metabolism crate for use with sheep. J. Animal Sci. 10:592.

Burton, G. W. and E. H. DeVane. 1952. Effect of rate and method of applying different sources of nitrogen upon the yield and chemical composition of Bermuda grass. Agronomy J. 44:128.

Ferguson, W. S. and R. A. Terry. 1954. The fractionation of the non-protein nitrogen of grassland herbage. J. Sci. Food Agr. 5:515.

Greenhill, A. W. 1936. A study of the relative amounts of protein and non-protein nitrogenous constituents occurring in pasture herbage. Biochem. J. 30:412.

Ramage, C. H. 1956. Yield and chemical composition of grasses and alfalfa-grass mixtures fertilized with heavy nitrogen and potash applications. Ph.D. Thesis Rutgers University, New Brunswick, N. J.

Ramage, C. H., R. E. Mather, C. Eby and E. R. Purvis. 1955. High protein grass by fertilization. J. Dairy Sci. 38:1410.

Salisbury, G. W., and F. B. Morrison. 1938. Early-cut, nitrogen-fertilized timothy hay as compared with alfalfa hay for feeding dairy cows. Cornell Agr. Exp. Sta. Bul. 694.

Swift, R. W., R. L. Cowan, R. H. Ingram, H. K. Maddy, G. P. Barron, E. C. Grose and J B. Washko. 1950. The relative nutritive value of Kentucky bluegrass, timothy, bromegrass, orchardgrass and alfalfa. J. Animal Sci. 9:363.

Wagner, R. E. 1954. Legume nitrogen versus fertilizer nitrogen in protein production of forage. Agronomy J. 46:233.

Woodman, H. E., R. E. Evans and D. B. Norman. 1933. Nutritive value of lucerne. J. Agr. Sci. 23:419.

THE EFFECTS OF PHOSPHATE FERTILIZATION OF ALFALFA ON GROWTH, REPRODUCTION, AND BODY COMPOSITION OF DOMESTIC RABBITS [1]

W. W. HEINEMANN,[2] M. E. ENSMINGER, W. E. HAM AND J. E. OLDFIELD
State College of Washington [3]

INTENSIVE agricultural practices in many of the western irrigated areas have reemphasized the need for adequate phosphorus fertilization of the soil in order to attain top yields of feed crops, especially legumes, containing optimum levels of phosphorus (Singleton *et al.*, 1945). Generally, western soils are low in available phosphorus. Beeson (1945) and Mitchell (1947) have pointed out that the most prevalent and widespread nutritional inadequacy among farm animals in the United States involves phosphorus. Beeson has identified the Pacific Northwest as being in a generalized soil-phosphorus deficient area.

McLean *et al.* (1944) reported varied rates and efficiency of gains corresponding to soil treatments. Singleton *et al.* (1945) found that phosphorus content of alfalfa was increased by 79% and the yield of hay by 50% through the application of phosphate fertilizer at an annual rate of 48 lb. per acre to phosphorus-deficient soils. This increase in phosphorus content far exceeds values reported in a review by Beeson (1946) for another soil type. This review concludes that phosphorus content can be increased slightly by use of phosphorus fertilizer, particularly where original phosphorus concentration in the plant is unusually low. It points out that phosphorus concentration in any species may be relatively resistant to change and that response to phosphorus application is dependent to a large degree on the nature of the soil.

Phosphorus content of the ration can be a limiting factor in the production of meat animals. Matrone *et al.* (1954b) have described a rabbit assay showing that rabbits fed forage not fertilized with phosphorus made smaller gains and had a lower level of inorganic phosphorus in the blood serum, and had bones of greater fragility, than did those fed diets containing forage that received phosphate fertilization. A widespread knowledge of the over-all benefits to be derived from the production and feeding of high phosphorus alfalfa hay should serve as a stimulus to the use of such alfalfa in producing more and better livestock. The writers are cognizant of the fact that increased phosphorus *per se* may not be the only altered characteristic of alfalfa hay produced on soil receiving phosphorus amendments.

[1] Scientific Paper No. 1510, Washington Agricultural Experiment Stations, Pullman. Project No. 810. The authors express appreciation to Dr. H. M. Krueger, Physiologist, Oregon State College, for reading the X-ray films.

[2] Data taken from a Ph.D. thesis submitted to Oregon State College by the senior author.

[3] Irrigation Experiment Station, Prosser, and Department of Animal Husbandry, Pullman.

Materials and Methods

The relationship of alfalfa hay grown on soils low or deficient in available phosphorus as compared with alfalfa grown under phosphorus fertilization was studied in domestic (New Zealand White) rabbits with respect to (a) growth and reproduction, (b) calcium, phosphorus, fat, and ash content of bones, (c) breaking strength and changes in calcification of bones, and (d) phosphorus content of the blood. A total of 538 animals (in four generations) were used in this study.

The soil on which all the alfalfa was grown is described as Sagemoor fine sandy loam, (Strahorn *et al.* 1929) a deep soil with laminated strata of silt loam and lime and having a pH of about 7.8.

The alfalfa hay was produced under controlled conditions of fertility, irrigation and harvesting. Annual applications of 66 lb. of available phos-

TABLE 1. AVERAGE PERCENTAGE COMPOSITION OF PELLETED RATIONS AND ALFALFA HAY

	Rations		Alfalfa hay	
	High P	Low P	High P	Low P
Moisture	5.96	5.89	6.56	6.39
Crude protein	11.81	10.89	13.00	9.70
Nitrogen-free extract	43.9	46.3	33.7	41.0
Crude fiber	28.0	28.1	36.2	35.4
Ash	9.11	7.94	9.07	6.54
Ether extract	1.22	0.97	1.45	0.98
Ca	1.50	1.50	1.48	1.20
P	0.18	0.12	0.26	0.12

phorus per acre constituted the only fertilizer amendment to the soil on which the high phosphorus hay was produced. Available soil moisture was not a limiting factor for either hay. Time of cutting for both hays was between tenth- and fourth-bloom stage. Good storage conditions were provided for the hay. As needed to compound pellets, equal quantities of the three cuttings of a given year were used.

Prior to being placed on the experimental rations, the original 16 females and 2 males were raised from weaning to six months of age on a commercial rabbit ration.

The experimental rations were identical except for the alfalfa hay. Components of the rations were: alfalfa, 86%; cane molasses, 9%; unenriched white wheat flour, 5%; carotene, 890 USP units per lb.; vitamin D_2, 750 USP units per lb.; thiamine hydrochloride, 0.72 mg. per lb.; alpha tocopherol, 1.00 mg. per lb. Data on average composition of the hays and pelleted rations are presented in table 1.

The feeding method was a modification of the "paired-feeding technique" (Mitchell and Beadles, 1930). By this practice, the feed intake among

mature does and among young animals of a given age was kept constant.

The animals selected for slaughter were females, with the exception of nine males which represented litters in which there were no females. The animal selected from each litter was the female (male in the nine non-female litters) having the heaviest body weight at weaning (8 weeks of age).

At the time of slaughter, a 30- to 40-ml. sample of blood was collected and immediately centrifuged at 2,000 r.p.m. in order to obtain a serum sample for the determination of total inorganic phosphorus. Immediately following the 10-minute centrifuging period, the serum was removed from the clot and stored in stoppered tubes at 5° C. Phosphorus determinations on the serum were made according to the method of Fiske and SubbaRow (1925), using an Evelyn colorimeter, on the same day the sample was obtained.

The left front leg of each animal slaughtered was used for the determinations on bone. Thus the humerus (brachium) was dissected from the carcass, using mechanical means for removing all the muscular and cartilagenous tissue. A modified recording fruit pressure tester with a 1/32" tip was used to determine breaking strength of the bone. The point of pressure for the breaking strength determinations was near the proximal end of the bone at a distance from the proximal end equal to the long lateral dimension of the joint at the proximal end of the humerus.

Ash, moisture and fat determinations of the bones were made according to methods or modifications of methods described in the Methods of Analysis of the A.O.A.C. (1950). Calcium determinations were made with the use of a Beckman DU spectrophotometer using a flame attachment.

Phosphorus content of the bone ash was determined according to a modification of the molybdivanadophosphoric acid method of Kitson and Mellon (1944).

Changes in ossification were measured by X-ray photography in individuals that were considered representative animals from either treatment. In most cases the X-ray pictures were taken of live animals, but pictures of front legs of comparable animals from either treatment were taken post-mortem.

Results and Discussion

Capacity for growth by animals in the litters produced by does on the two treatments differed greatly. Analysis of variance of the weaning weights revealed a significant difference ($P<0.01$) between the two treatments (table 2). Post-weaning gains, as measured by weighings at 5 months of age, were greater for the animals receiving the high phosphorus ration. Five-month average weights for the animals on growth trials were 6.07 and 5.15 lb. respectively for the high and low treatments. Likewise, average daily gains were .043 and .034 lb. per day both of which are lower than

normal for well nourished rabbits. The differences were not significant ($P>0.05$). The deficits in body weights of does on the untreated hay were never overcome, resulting in lowered mature weights. Some of this effect could have been due to the 8% higher protein content of the high phosphorus ration. Eckles and Gullickson (1928) observed similar results with dairy animals. Matrone *et al.* (1954a) noted statistically significant increases in rates of gain with younger weaned rabbits on a shorter feeding period.

More services were required per conception for the does on the low phosphorus ration than for the does on the high phosphorus ration. Beeson *et al.* (1945) reported similar findings with sheep. Services per conception

TABLE 2. MEANS OF WEANING WEIGHTS

Phosphorus level	Year	Male		Female		Weighted mean, both sexes
		No.	Mean wt.	No.	Mean wt.	
			lb.		lb.	lb.
Low	1950	30	1.69	45	1.72	1.71
Low	1951	33	1.74	33	1.74	1.74
Low	1952	39	1.85	43	1.80	1.82
Low	3 years	102	1.77	121	1.75	1.76
High	1950	56	1.91	53	1.78	1.84
High	1951	46	1.85	48	1.99	1.92
High	1952	44	2.15	30	2.16	2.15
High	3 years	146	1.96	131	1.94	1.95
Weighted Av.			1.88		1.85	
Difference			+0.19		+0.19	+0.19**

** $P<0.01$.

over the three-year period averaged 1.67 and 1.47, respectively. Because the breeding data approach a Poisson distribution, (Snedecor, 1946) they were converted to $\sqrt{x + 0.5}$ for statistical analysis. An analysis of variance showed that a significant difference ($P<0.01$) existed between the conception rates of the does on the two treatments. The does on either treatment seemed to mate readily, and dietary regimen appeared to have no diminutive effect on sexual function.

Number of animals born per litter was not greatly different between treatments. Likewise, litter size at weaning was approximately equal. Therefore, once the does became pregnant and carried a litter to term, the litters seemed to have about the same chance for survival on either treatment.

Under the conditions of this experiment, level of phosphorus in the ration had no effect on the phosphorus content of the blood serum ($P>0.05$) (table 3). There was some variation among years within levels, primarily

TABLE 3. MEANS OF BONE AND BLOOD DETERMINATIONS OF 10-WEEK-OLD ANIMALS

Phosphorus level	Year	Bone analyses: Moisture-free basis Ash	Fat	Ca	P	Breaking strength	Serum phos.
		%	%	%	%	lb.	mg. %
Low	1950	52.00	22.07	7.26	6.81	6.64	11.99
Low	1951	51.05	20.33	7.68	6.73	6.93	11.43
Low	1952	50.92	22.90	7.27	6.62	5.57	11.43
Low	3 years	51.18	21.85	7.41	6.70	6.26	11.65
High	1950	43.04	17.77	5.70	6.15	9.22	11.08
High	1951	43.24	16.63	6.38	6.15	11.67	11.99
High	1952	43.74	16.91	5.89	6.15	10.96	12.00
High	3 years	43.33	17.05	6.04	6.15	10.73	11.57
Weighted av.		46.68	19.10	6.62	6.39	8.79	11.60
Difference		—4.85**	—2.80**	—1.37**	—0.55**	+4.47**	0.08

** $P<0.01$.

in animals fed the treated hay the first year and in those fed the untreated hay the third year ($P<0.05$). Any explanation for these small variations would be conjecture. The total inorganic phosphorus level of the serum of these animals was considerably higher than reported by Matrone *et al.* (1945b) for rabbits 5 weeks older than those used in this work. Also, Matrone *et al.*, observed a positive correlation between phosphorus content of the diet and inorganic phosphorus level in the serum.

The relatively high and uniform level of inorganic phosphorus in the serum among animals of both treatments is not readily explainable except that non-oxalated samples were used. Albritton (1955) reported values in a range of 3.4 to 6.9 mg. per 100 ml. of oxalated blood. Other animals

TABLE 4. MEANS OF BONE DETERMINATIONS OF MATURE DOES

Phosphorus level	Bone analyses: Moisture-free basis Ash	Fat	Ca	P	Breaking strength
	%	%	%	%	lb.
Low	50.2	11.82	8.05	8.61	7.21
High	46.6	18.98	7.06	7.74	13.58
Difference	—3.6*	+7.16**	—0.99*	—0.87*	+6.37**

* $P<0.05$; ** $P<0.01$.

have shown a lowering of inorganic blood phosphorus when a low phosphorus (0.20% phosphorus) ration was fed (Huffman *et al.*, 1933). It would appear that the blood of rabbits on a low phosphorus ration is not affected in the same way as that of many other species.

The content of phosphorus in the bones of rabbits in the present work was generally within the same range of values reported by Åslander (1952). However, there was a highly significant difference between treatments in both calcium and phosphorus content, although all of the calcium values were considerably lower than reported in the above reference.

Very marked increases in organic matter and moisture had occurred in the bones of the animals fed the untreated hay and killed at 10 weeks of age. Howland *et al.* (1926) reported that in rickets the amount of minerals laid down is always greatly diminished, and the water, fat and organic matter are proportionately greater than in normal. This is in agreement with work reported by Dutcher *et al.* (1925). Thus, it would appear that rickets was not a factor in the present work. However, in the mature does there was less fat in the bones of mature animals fed the untreated hay (table 4). Also, in the work of the present experiment, both the calcium and phosphorus contents of the bones were decidedly increased in the low phosphorus animals on the basis of percentage of dried bone weight and on the basis of total weight of calcium and phosphorus.

On the dry basis, bones from animals receiving the low phosphorus hay and killed at 10 weeks of age had a significantly higher ($P<0.01$) percentage of fat than bones from the animals receiving the high phosphorus hay (table 3). Likewise, the ash content, on the basis of vacuum-dried bones, was significantly greater for the animals on the untreated hay (table 3). In the case of either fat and ash, there was no significant difference attributed to among years within levels of phosphorus nutrition. Thus, the fat and ash contents remained relatively fixed from one year to the next.

Calcium content of the bones of 10-week-old animals, on a moisture-free basis, was significantly different between the two levels (table 3). The difference among years within phosphorus levels was significant at the 5% level. However, the important bone characteristic insofar as calcium is concerned is the fact that the animals receiving the low phosphorus hay produced bones with significantly greater quantities of calcium. Analysis of variance calculations on the basis of the actual weight of calcium indicate a significant difference for between treatments ($P<0.05$) and for among years variation ($P<0.01$). Also, the calcium content of the bones from animals fed the treated hay varied considerably less.

As with calcium content of the bones, the phosphorus content was significantly lower ($P<0.01$) on a percentage and on a total moisture-free weight basis in bones from the 10-week-old animals receiving the high phosphorus hay (table 3). Also, there appeared to be more variation within the low treatment than within the high treatment.

Analysis of the bones of mature does showed significant differences

between levels in both calcium and phosphorus content. The differences showed significance at the 5% level (table 4). Generally the bones from does fed the untreated hay contained higher percentages of calcium and phosphorus, respectively, than bones from does fed the treated hay. This corresponds with the data obtained from the animals killed at 10 weeks of age. However, bones of mature does on the low treatment contained less fat than bones of mature does on the high treatment.

The calcium:phosphorus ratio of the bones (humerus) of 10-week-old animals was significantly altered by the dietary treatments ($P<0.01$), with means of 1:0.98 and 1:1.07 for high and low treatments, respectively.

Mitchell (1947) observes that "it is indeed strange, in view of the importance of phosphorus to the basic phenomena of animal life, that the stores of phosphorus in the skeleton are so difficult to tap when the need arises. When there is a calcium deficiency in the food supply, the calcium phosphate of the bones is readily mobilized to maintain the calcium content of the blood and tissues. The hormone of the parathyroid glands is a specific mobilizing agent for calcium. No such agent exists for phosphorus." Although there is a tremendous store of phosphorus in the bones, the animal lives on a current basis as far as this element is concerned. Thus a phosphorus deficiency develops relatively quickly. In the present work a significant difference in phosphorus content of the bones existed between treatments, which would indicate a lower percentage level of phosphorus storage in the bones.

The test of breaking strength of bones revealed very marked differences between bones from young animals as well as mature does on the two kinds of hay (tables 3 and 4). Also, it is worthy of note that the differences became more distinct and the means were further apart with each succeeding year among the 10-week-old animals. Thus, in 1952, for example, the highest reading or greatest breaking strength for these animals on the low phosphorus hay was 7.15 lb., and the lowest breaking strength among the animals on the high phosphorus hay was 8.50 lb. Hall *et al.* (1944) found that a phosphorus deficient ration fed to steers did not cause a decreased strength of bones.

X-ray photographs of six live animals, three from each treatment, and the front legs of four animals, two from each treatment, revealed several distinct changes in calcification in bones of the animals on the low phosphorus treatment. The vertebrae of animals on the high phosphorus hay were much thicker and more dense than in the animals on the ration containing the low phosphorus hay. The vertebral processes in the former were much more clearly defined. Also, the posterior processes at the insertion of the femur into the pelvis were much more prominent in animals on the high phosphorus alfalfa. The rib shafts appeared to be thicker in these animals. X-rays of their skeletons also revealed a wider head on the femur and humerus. Generally, there was much less X-ray absorption in the negatives of the animals fed the low phosphorus hay, suggesting softer

bones. This assumption is not without basis. Two mature does (one from either treatment) killed at the same age, were found to have widely different breaking strength of bones. Thus, a doe fed the fertilized alfalfa had a breaking strength of over 20 lb. on the humerus and a doe fed the untreated hay had a breaking strength of only 8.00 lb. on the humerus.

In other comparisons of X-ray pictures of animals on the two levels of treatment, it was observed that without exception more X-rays were absorbed by the bones of the animals that received the fertilized hay. Other characteristics observed were thicker vertebrae, more prominent vertebral spines, and better calcification of joint connective tissues in the skeletons of animals fed the treated hay.

Summary

Domestic rabbits were used to determine effects of alfalfa hay produced on soil with (1) low available phosphorus and (2) high available phosphorus on (a) efficiency of reproduction, (b) growth rate, (c) inorganic phosphorus content of blood serum, (d) breaking strength and calcification of skeletons, and (e) calcium, phosphorus, fat, and total ash of bones. Alfalfa hays containing 0.12% and 0.26% phosphorus were used as 86% of the rations. Differences observed as the result of feeding low phosphorus hay included significantly retarded growth, lower mature body weights, impaired breeding efficiency, significantly higher levels of calcium, phosphorus, fat, and ash in the skeletons of 10-week-old animals and significantly lower levels of ash in skeletons of mature animals, extremely low breaking strength of the humerus, approaching 100% difference in the third and fourth generations, and abnormal bone structure and ossification as revealed by X-ray photographs. There was no change in inorganic phosphorus in the serum. It is concluded that where the phosphorus content of alfalfa hay produced on a soil of low available phosphorus is of the order of 0.12%, it is not adequate for normal rabbit nutrition. Increased skeletal strength, improved breeding efficiency and increased rate of growth can be effected by proper phosphate fertilization of the soil. Results of this study establish that there is a positive soil-plant-animal relationship among (a) the soil on which alfalfa hay was produced for this work, (b) the forage crop produced thereon, and (c) the animal organism.

Literature Cited

Albritton, E. C. 1955. Standard Values in Blood. Saunders, Philadelphia.

Åslander, A. 1952. Standard fertilization and the quality of crops. Soil Sci. 74:431.

Association of Official Agricultural Chemists. 1950. Official Methods of Analysis. 7th Ed. Banta, Washington.

Beeson, K. C. 1945. The occurrence of mineral nutritional diseases of plants and animals in the United States. Soil Sci. 60:9

Beeson, K. C. 1946. The effect of mineral supply on the mineral concentration and nutritional quality of plants. Botanical Rev. 12:424.

Beeson, W. M., C. W. Hickman and R. F. Johnson. 1945. Calcium and phosphorus for breeding ewes. Flour and Feed 46:12.

Dutcher, R. A., M. Creighton and H. A. Rothrock. 1925. Vitamin studies XI. Inorganic blood phosphorus and bone ash in rats fed on normal, rachitic and irradiated rachitic diets. J. Biol. Chem. 66:401.

Eckles, C. H. and T. W. Gullickson. 1927. The relation of phosphorus deficiency to the utilization of feed. Proc. Amer. Soc. Animal Prod. 16.

Fiske, C. H. and Y. SubbaRow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375.

Hall, J. G., D. L. Mackintosh and G. E. Vail. 1944. Quality of beef. Kans. Agr. Exp. Sta. Tech. Bul. 58.

Howland, J., W. McK. Marriott and B. Kramer. 1926. Studies upon the inorganic composition of bones. J. Biol. Chem. 68:721.

Huffman, C. F., C. W. Duncan, C. S. Robinson and L. W. Lamb. 1933. Phosphorus requirement of dairy cattle when alfalfa furnishes the principal source of protein. Mich. Agr. Exp. Sta. Tech. Bul. 134.

Kitson, R. E. and M. G. Mellon. 1944. Colorimetric determinations of phosphorus as the molybdivanadophosphoric acid. Ind. & Eng. Chem., Anal. Ed. 16:379.

Matrone, G., F. H. Smith, V. B. Weldon, W. W. Woodhouse, Jr., W. J. Peterson and K. C. Beeson. 1954a. Effect of phosphate fertilization on the nutritive value of soybean forage for sheep and rabbits. U.S.D.A. Tech. Bul. 1086.

Matrone, G., V. B. Weldon, W. W. G. Smart, Jr., F. W. Sherwood, F. H. Smith and G. H. Wise. 1954b. Effects of phosphate fertilization and dietary mineral supplements on the nutritive value of soybean forage. J. Nutr. 52:127.

McLean, E. O., G. E. Smith and W. A. Albrecht. 1944. Biological assays of some soil types under treatments. Proc. Soil Sci. Soc. Amer. 8:283.

Mitchell, H. H. 1947. Mineral requirements of farm animals. J. Animal Sci. 6:365.

Mitchell, H. H. and J. R. Beadles. 1930. The paired-feeding method in nutrition experiments and its application to the problem of cystine deficiencies in food proteins. J. Nutr. 2:225.

Singleton, H. P., C. E. Nelson and C. O. Stanberry. 1945. Fertilizers for irrigated alfalfa. Wash. Agr. Exp. Stas. Bul. 465.

Snedecor, G. W. 1946. Statistical Methods. 4th Ed. Iowa State College Press, Ames.

Strahorn, A. T., E. J. Carpenter, W. W. Weir, S. Ewing and H. H. Krusekopf. 1929. Soil survey (reconnaissance) of the Columbia Basin area Washington. U.S.D.A. No. 28. (Series 1929).

THE DIGESTIBILITIES OF INTERIOR LIVE OAK AND CHAMISE BY DEER AND SHEEP

HAROLD D. BISSELL [1] AND WM. C. WEIR [2]
University of California, Davis

SHEEP and deer occupy the same ranges in many places in the Coastal chaparral of Northern California. The food items in the diets of these animals include the same species at certain seasons.

Rumen samples from 139 deer and 68 sheep taken on Hopland Field Station in southeastern Mendocino County by William M. Longhurst and analyzed by Howard Leach of the Department of Fish and Game indicate the chamise (*Adenostoma fasiculatum*) and interior live oak (*Quercus wislizenii*) are among the most important browse species for both animals. Deer ate these species in all seasons with heaviest use in the spring and summer and sheep ate them primarily during the late spring and summer months.

Digestion trials using both animals on the same rations were devised in an attempt to compare efficiency of digestion and obtain a more accurate knowledge of the value of these browse species in planning management of a chaparral area.

The digestion trials with deer were carried out by using Department of Fish and Game facilities at the Yountville Game Farm while the Department of Animal Husbandry of the University of California, Davis, conducted the digestion trials with sheep.

Experimental

Browsing animals normally select only the current year's growth from shrubs. For these trials an attempt was made to select only the portion animals have been observed to eat. This new growth of chamise and interior live oak was collected during late May, 1954, from brush stands on Cow Mountain in Lake County close to the boundary of Mendocino County. This plant material was sacked in burlap bags and later spread in a thin layer on cloth on a drying room floor. The material was dry enough to chop after air-drying in the shed for one week. Uniform field-cured alfalfa hay grown at the University was chopped for the trial. All chopped material was later ground to a fine meal in a hammer mill.

Since previous digestion and feeding trials (Bissell *et al.*, 1955) had demonstrated the unpalatability of a straight chamise or live oak diet for

[1] Game Manager II, Pittman-Robertson Project 1Kw25R, California Department of Fish and Game, Sacramento, California.

[2] Department of Animal Husbandry. Acknowledgement is made for technical assistance to William Schafer and Bruce Harris of the Department of Fish and Game and A. R. Terrill of the Department of Animal Husbandry. Special appreciation is given those Fish and Game personnel from Fish and Game Region III who assisted in collecting the browse.

captive deer, the experiments were designed to determine the digestibility of chamise and live oak by difference when mixed with alfalfa. Approximately 50–50 mixtures were made of chamise plus alfalfa and interior live oak plus alfalfa. These mixtures and the alfalfa alone were pelleted by a commercial mill.

Deer Digestion Trials. A 3 x 3 latin square digestion trial was conducted using three yearling Columbian black-tail deer (*Odocoileus hemionus columbianus*). They had been fed in captivity on barley and alfalfa since weaning. Two deer were males and one was a stag. One deer developed an infection during the second period and was replaced by another male black-tail.

Three digestion trial cages were constructed as reported by Bissell *et al.*, (1955). These are essentially 4 foot cubes with the animals standing on one half inch mesh, 9 gauge galvanized wire screen. Feces fell through the floor and were deflected into a collecting tray by an inclined strip of

TABLE 1. CHEMICAL COMPOSITION OF FEEDS (DRY BASIS)

Feed	Ash	Protein	Crude fiber	Ether extract	Nitrogen-free extract (NFE)
	%	%	%	%	%
Alfalfa hay	9.1	19.9	26.6	1.2	43.2
Chamise	3.3	7.1	20.7	5.4	63.6
Live oak	3.1	7.4	27.4	1.4	60.7

ordinary window screening which allowed urine to pass through with a minimum of contamination of the feces.

The deer were fed at the maintenance level of 2.5 lb. of oven dry material per 100 lb. of live weight daily. This figure was obtained from previous deer digestion trials and from livestock data. Since the pelleted food was about 10% water, the actual amount fed was about 2.7 lb. per 100 lb. of animal. A 7-day preliminary period and a 7-day collection period was used.

Sheep Digestion Trials. A 3 x 3 latin square digestion trial was conducted using three yearling wethers in conventional sheep collection stalls. The sheep were fed at an estimated maintenance level of 2.2 lb. of air-dry fed per 100 lb, body weight daily. A 7-day preliminary period and a 7-day collection period was used.

Table 1 summarizes the chemical analyses of the feeds used.

Results

The results of the digestion trials are given in table 2. For deer total digestible nutrients (TDN) were calculated using three different methods, conventional, calorimetric (Lofgreen, 1951) and the method based on

digestible organic matter described by Lofgreen (1953). For sheep only the conventional and Lofgreen TDN were calculated. All digestibilities of chamise and live oak were obtained by difference.

Although there are some differences, in general, the feeds and the various fractions of the feeds were digested similarly by both the deer and the sheep.

The organic matter of the alfalfa and live oak was more highly digested by sheep than by deer but with chamise there was little difference. The crude protein (N x 6.25) of the alfalfa was well digested by both the sheep and deer, but the chamise protein was digested only about half as well.

TABLE 2. DIGESTION COEFFICIENTS AND TOTAL DIGESTIBLE NUTRIENTS

Feed		Alfalfa hay		Chamise		Live oak	
Animal		Deer	Sheep	Deer	Sheep	Deer	Sheep
Organic matter	%	59.3	63.3	53.8	56.6	42.6	52.8
	S	(2.1)	(0.5)	(5.4)	(4.0)	(3.0)	(2.5)
Protein	%	74.9	77.4	37.1	30.0	10.7	—.11
	S	(1.9)	(0.6)	(14.3)	(14.3)	(18.6)	(.13)
Crude fiber	%	37.9	39.7	20.8	12.6	9.9	20.4
	S	(2.5)	(1.6)	(20.7)	(12.0)	(17.1)	(19.0)
Ether extract	%	57.2	16.1	48.1	26.4	33.4	46.5
	S	(5.3)	(6.6)	(10.6)	(5.7)	(29.4)	(27.9)
Nitrogen-free-extract	%	66.2	72.6	57.6	63.4	58.4	55.5
	S	(4.5)	(0.4)	(6.7)	(3.6)	(4.8)	(3.3)
Conventional TDN	%	58.4	57.5	47.8	48.2	37.5	41.2
(dry basis)	S	(2.2)	(0.5)	(1.1)	(5.9)	(2.1)	(6.0)
Lofgreen TDN	%	55.5	58.5	53.4	48.5	40.7	41.3
(dry basis)	S	(1.7)	(0.5)	(5.4)	(8.2)	(2.9)	(5.0)
Calorimetric TDN	%	51.9		50.7		40.8	
	S	(2.5)		(1.6)		(2.5)	

S=Standard deviation.

The protein fraction of the live oak was very poorly digested by the deer and with sheep negative digestibilities were obtained with all animals.

The crude fiber of the alfalfa was digested equally well by both the sheep and deer, but the crude fiber portion of the two browse species was poorly digested by both sheep and deer with large variations in digestibility noted. This might be due to differing amounts of woody stems in the rations of the various animals from incomplete mixing, however, care was exercised in obtaining the browse to collect only the current year's growth. The ether extract digestibilities vary considerably but the amount present in the alfalfa and live oak was so small as to be of little nutritional consequence. The 5.4% ether extract of the chamise was large enough to influence the TDN result. Further study to determine the true nature of this ether extract should be conducted as Cook *et al.* (1952) have found that

most of the ether extract portion of browse species may be excreted in the urine. The nitrogen-free extract portion of these feeds appears to be about equally digested by both species.

There appears to be no significant difference in the three methods used for calculating TDN, since in nearly every case the standard deviations allow an overlap of error.

Chamise TDN is only slightly lower than that obtained from alfalfa hay. Live oak TDN was significantly lower than alfalfa.

Possible sources of error in these experiments include incomplete mixing of pellet components, failure or lag on the part of rumen microorganisms in adapting to chamise and oak as a substrate, loss of small amounts of feed or feces in digestion trial cages, unavoidable occasional contamination of feces with urine. The determination of the digestibility of the two browse species by difference with alfalfa as the base feed makes it impossible to determine any influence the alfalfa may have had on the digestion of the browse.

Blaxter *et al.* (1956) have reported that grinding and cubing of dried grass reduced the digestibility compared to the chopped grass. This effect was particularly marked with the crude fiber fraction. As all rations used in this study were ground and pelleted, the digestion coefficients may be lower than would be expected with chopped forage.

In general, however, it appears that alfalfa hay is equally well utilized by deer and sheep and that chamise serves as a reasonably good source of TDN as well as contributing some digestible protein. Live oak is of little, if any, value as a protein feed and its crude fiber is poorly digested.

It thus appears that deer and sheep on a chaparral range would obtain some energy from chamise and interior live oak in May and June, but that protein should be supplied from other sources.

Summary

Deer and sheep digested the protein of alfalfa hay distinctly better than the protein of chamise or interior live oak. Chamise was considerably superior to interior live oak in regards to protein digestibility. The percent of TDN is high in alfalfa, slightly less in chamise, and interior live oak distinctly poorer. It appears that chamise is a good feed in supplying TDN and some of the protein requirements. Interior live oak appears to be of little or no value as a source of crude protein, although a fair source of TDN.

Little difference was noted between sheep and deer in their ability to utilize alfalfa, chamise or live oak.

References Cited

Blaxter, K. L. and N. McC Graham. 1956. The effect of the grinding and cubing process on the utilization of the energy of dried grass. J. Agr. Sci. 47:207.

Cook, C. W., L. A. Stoddart and L. E. Harris. 1952. Determining the digestibility and metabolizable energy of winter range plants by sheep. J. Animal Sci. 11:578.

Bissell, H. D., B. Harris, H. Strong and F. James. 1955. The digestibility of certain natural and artificial foods eaten by deer in California. Calif. Fish & Game, 41:57.

Lofgreen, G. P. 1951. The use of digestible energy in the evaluation of feeds. J. Animal Sci. 10:344.

Lofgreen, G. P. 1953. The estimation of total digestible nutrients from digestible organic matter. J. Animal Sci. 12:359.

A COMPARISON OF THE LIVE PROBE AND LEAN METER FOR PREDICTING VARIOUS CARCASS MEASUREMENTS OF SWINE [1]

A. M. Pearson, J. F. Price, J. A. Hoefer, L. J. Bratzler and W. T. Magee [2]

Michigan Agricultural Experiment Station [3]

IN 1952, Hazel and Kline reported on the development of the live probe technique for measuring backfat thickness. Since that time, this method has been widely used as an aid in selection of swine for breeding purposes. More recently the lean meter, the operation of which is based on the difference in electrical conductivity between fat and lean tissues, was developed by Andrews and Whaley (1954). Thus, the lean meter has in part supplemented the live probe for measuring backfat thickness. However, there is no information available in the literature comparing the two methods. As a result, this study was undertaken to compare the two methods from the standpoint of both physical carcass measurements and carcass cut-outs.

Materials and Methods

Experimental Animals. A total of 99 hogs were used in this study. The pigs were raised under various feeding regimes; some coming from feeding experiments, while the others were from the Michigan Swine Evaluation Station. A total of 43 gilts and 56 barrows, representing the Duroc, Chester White, Poland China, Tamworth and Yorkshire breeds and Chester White-Duroc crossbred pigs were used in this study. No attempt was made to segregate the pigs on a basis of breed or nutritional background.

Probing Procedure. Each hog was marked with chalk across the top of the back directly above the point of the elbow, at a point directly above the stifle joint and halfway between the other two points. Probes were made at each chalk mark 1.5 inches off the midline on each side. The six readings for each method were averaged for all subsequent statistical comparisons. Lean meter readings were taken first to eliminate the possibility of interference by bleeding from the incision in making the live probe.

Other Measures of Leanness or Fatness. Dressing percentage, carcass length, backfat thickness, lean cuts (both live and carcass basis), primal cuts (both live and carcass basis), specific gravity of the ham, area of the *Longissimus dorsi* muscle at both the 10th and last rib and percent fat trim were obtained according to the procedure outlined by Pearson *et al.* (1956a).

[1] Journal Article 1989 Michigan Agricultural Experiment Station, East Lansing.
[2] Department of Animal Husbandry.
[3] The authors acknowledge the assistance of Mr. E. C. Miller in the initial work using the lean meter.

Results and Discussion

Examination of the correlation between the live probe and lean meter (table 2) revealed the relationship was statistically significant at the 1% level (r=+0.78). However, the variation in one measurement accounted for less than 62% of the variation in the other. Both methods were designed to measure backfat thickness, and are apparently about equally accurate in this respect, with correlation coefficients of +0.70 and +0.71 for the live probe and lean meter, respectively. The degree of association between dressing percentage and live probe was very low and was not statistically significant. However, the correlation coefficient for dressing percentage with

TABLE 1. MEANS AND STANDARD DEVIATIONS FOR HOG CARCASSES

	Mean	Standard deviation
Slaughter weight, lb.	201.0	21.4
Live probe, in.[a]	1.81	0.21
Lean meter, in.[a]	1.89	0.22
Dressing percentage [b]	75.20	1.45
Carcass length, in.[c]	28.74	1.18
Backfat, average, in.[d]	1.81	0.18
Lean cuts, live, %	35.96	2.19
Lean cuts, carcass, %	47.82	2.84
Primal cuts, live, %	46.96	2.06
Primal cuts, carcass, %	62.36	2.73
Specific gravity, right ham	1.044	0.0075
Area *Longissimus dorsi,* last rib	3.72	0.60
Area *Longissimus dorsi,* 10th rib	3.50	0.52
Fat trim, % [e]	26.09	2.69

[a] Determined at three locations on the live hog—approximately at 5th rib, last rib and last lumbar. One reading on each side of the midline at each location.
[b] Based on shrunk live weight and cold carcass weight.
[c] Carcass length—anterior edge 1st rib to anterior edge of aitch bone.
[d] Average backfat—measured at 1st rib, 7th rib, last rib and last lumbar vertebra.
[e] Based on cold carcass weight.

the lean meter (r=+0.21) was significant at the 5% level. Close examination of the data revealed the lean meter was no more accurate in predicting dressing percentage of the gilts than was the live probe, but showed a higher degree of association for the barrows (r=+0.25). Since all correlation coefficients were low, the difference in favor of the lean meter is of doubtful significance. Both live probe and lean meter measurements were negatively correlated with carcass length, with both being significant at the 1% level. However, the live probe showed a consistently higher relationship for both barrows and gilts as well as for the two sexes combined. The relationship between the live probe and carcass length (table 2) was low, which is in agreement with results reported by Pearson *et al.* (1955b).

The relationship between lean cuts on either the carcass or live basis differed little between the live probe or lean meter (table 2). The relation-

ships were of the magnitude of —0.62 on the live basis and —0.67 on the carcass basis. Primal cuts on the live basis did not differ greatly for either the live probe (r=—0.67) or the lean meter (r=—0.68). However, examination of the data in table 2 showed that live probe was considerably higher for primal cuts on the carcass basis (r=—0.52) with the gilts than was the case for the lean meter (r=—0.23).

Comparison of the correlation coefficients for the lean meter and the live probe with the area of the *Longissimus dorsi* muscle at the last rib (table 2) showed the relationship was consistently higher for the live probe. The corresponding values for the area at the 10th rib were likewise consistently higher. Furthermore, a higher relationship existed with the lean area of the 10th rib for both the lean meter and live probe than was the case for the last rib. This verifies the results reported for the live probe by Price *et al.* (1957).

TABLE 2. CORRELATION COEFFICIENTS FOR THE LEAN METER AND LIVE PROBE WITH VARIOUS CARCASS MEASUREMENTS

	Live probe			Lean meter		
	Barrows	Gilts	Combined	Barrows	Gilts	Combined
Lean meter	+0.78**	+0.71**	+0.78**	....	....	....
Dressing percentage	+0.10	—0.02	+0.11	+0.25*	+0.02	+0.21*
Carcass length	—0.20*	—0.43**	—0.38**	—0.10	—0.34**	—0.28**
Backfat thickness	+0.64**	+0.74**	+0.70**	+0.66**	+0.72**	+0.71**
Lean cuts, live basis	—0.60**	—0.55**	—0.62**	—0.59**	—0.52**	—0.60**
Lean cuts, carcass basis	—0.64**	—0.59**	—0.67**	—0.69**	—0.57**	—0.68**
Primal cuts, live basis	—0.56**	—0.61**	—0.61**	—0.58**	—0.47**	—0.57**
Primal cuts, carcass basis	—0.61**	—0.52**	—0.61**	—0.67**	—0.23**	—0.54**
Specific gravity of ham	—0.43**	—0.67**	—0.56**	—0.47**	—0.57**	—0.53**
Lean area, last rib	—0.33**	—0.65**	—0.49**	—0.12	—0.35**	—0.28**
Lean area, 10th rib	—0.42**	—0.70**	—0.58**	—0.32**	—0.40**	—0.40**
Fat trim	+0.68**	+0.65**	+0.67**	+0.56**	+0.42**	+0.52**

* Significant at 5% level.
** Significant at 1% level.

The association of fat trim with the live probe was higher (table 2) than with the lean meter. Although the differences were not large, the consistently higher relationship for the two sex groups and the two sexes combined indicated that live probe more accurately measured percentage fat trim than the lean meter.

Summary

Results indicate that there was little difference in the usefulness of the live probe or lean meter in regard to estimating backfat thickness and percentage of either lean or primal cuts. However, the higher relationship for the live probe with both loin lean areas and with fat trim indicated live probe to be a more reliable measure for estimating carcass leanness.

References Cited

Andrews, F. N. and R. M. Whaley. 1954. A Method for the Measurement of Subcutaneous Fat and Muscular Tissues in the Live Animal. Purdue Univ., Lafayette, Indiana.

Hazel, L. N. and E. A. Kline. 1952. Mechanical measurement of fatness and carcass value on live hogs. J. Animal Sci. 11:313.

Pearson, A. M., L. J. Bratzler, R. J. Deans, J. F. Price, J. A. Hoefer, E. P. Reineke and R. W. Luecke. 1956a. The use of specific gravity of certain untrimmed pork cuts as a measure of carcass value. J. Animal Sci. 15:86.

Pearson, A. M., L. J. Bratzler, J. A. Hoefer, J. F. Price, W. T. Magee, and R. J. Deans. 1956b. The fat-lean ratio in the rough loin as a tool in evaluation of pork carcasses. J. Animal Sci. 15:896.

Price, J. F., A. M. Pearson and E. J. Benne. 1957. Specific gravity and chemical composition of the untrimmed ham as related to leanness of pork carcasses. J. Animal Sci. 16:85.

SPLITTING AND CUTTING ERRORS IN SWINE CARCASS EVALUATION [1]

E. L. LASLEY [2] AND E. A. KLINE
Iowa State College

THE objectives of this investigation were to determine the splitting and cutting errors (including errors in measuring the various items), to identify the wholesale cuts or carcass traits most subject to error and to compare the reliability of carcass appraisals based on one or both sides.

Materials and Methods

Data were provided by 222 barrow carcasses slaughtered in the Iowa State College Meats Laboratory during three different seasons. Several breeds or crosses were included in each season but no attempt was made to identify genetic differences. The carcass weight averaged 155 lb. and ranged from 140 to 168. The standard deviation within seasons was 3.9 lb. Computations were made on absolute weights of cuts and were carried out within seasons because seasonal differences existed for most traits.

Both sides of each carcass were cut following the procedure outlined in the Fourth Annual Reciprocal Meat Conference Proceedings (1951). The same two persons, each performing certain tasks, cut most of the carcasses. Occasionally one person was replaced by a third. No attempt was made to identify variation due to such changes in personnel.

Traits investigated include weight (pounds) of half carcass, trimmed belly, loin, picnic, Boston butt, skinned ham, four lean cuts and five primal cuts; carcass length from front of first rib to aitch bone (centimeters); average of four backfat measurements (centimeters); loin eye area at tenth rib (square inches) for 203 barrows; and loin eye area at last rib for 122 barrows. Loin eye area was measured by planimeter from an outline tracing of the *Longissimus dorsi*. In most cases the area was measured once and the results recorded to one-tenth square inch. However, three determinations were made at both tenth rib and last rib for 18 carcasses and the average recorded to one-hundredth square inch.

Results and Discussion

The means for left and right sides, mean difference between sides and standard deviation of differences for each carcass trait are summarized in table 1. The mean differences between left and right sides are statistically

[1] Journal paper No. J-3059 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 1127. The project is cooperative with the U.S.D.A., A.R.S., Animal & Poultry Husbandry Research Branch, Regional Swine Breeding Laboratory, Ames, Iowa.

[2] Present address: Dept. of Animal Science, University of Illinois, Urbana.

significant ($P<.01$) for all traits measured by weight. The left side averaged heavier; yielded heavier loin, ham, picnic, lean cuts and primal cuts but lighter belly and Boston butt. In these data, consistent evaluation of either left or right side produces biased values for all these traits. For example, the weight of lean cuts from the left side is .375 lb. greater than the average weight of lean cuts from both sides. Linear and area measurements do not differ appreciably.

The variances of cutting errors are given in table 2. These values were computed as the pigs x sides interaction and are identical to one-half the variance of differences between sides after removal of the average left-right

TABLE 1. MEANS, AVERAGE LEFT-RIGHT DIFFERENCES AND STANDARD DEVIATIONS OF DIFFERENCES BY TRAITS [a]

Trait	Left mean	Right mean	Average left-right difference	St. dev. of difference
Half carcass, lb.	78.19	77.22	.97 [b]	2.40
Belly, lb.	10.70	10.94	—.24 [b]	.68
Loin, lb.	11.07	10.91	.17 [b]	.85
Ham, lb.	13.10	12.91	.19 [b]	.47
Picnic, lb.	7.48	7.00	.47 [b]	.67
B. butt, lb.	4.48	4.56	—.08 [b]	.42
Lean cuts, lb.	36.13	35.38	.75 [b]	1.36
Primal cuts, lb.	46.83	46.32	.51 [b]	1.38
Length, cm.	75.29	75.24	.05	.41
Av. backfat, cm.	4.328	4.342	—.014	.13
Loin eye area, tenth rib, sq. in.	3.335	3.360	—.025	.25
Loin eye area, last rib, sq. in.	3.484	3.506	—.022	.23

[a] Number of barrows: Loin eye area, tenth rib, 203; Loin eye area, last rib, 122; all other traits, 222.

[b] Statistically significant ($P<.01$).

difference. The variances for loin, ham, picnic and Boston butt sum to .635 while the variance for lean cuts is .649. This discrepancy could be caused by rounding errors or correlations between cutting errors or both. The magnitude of cutting errors for lean cuts are: loin, 53%; ham, 14%; picnic, 18%; Boston butt, 13%; and joint effects plus rounding errors, 2%. Similarly, the various sources of cutting errors for primal cuts are: belly, 24%; loin, 42%; ham, 11%; picnic, 14%; Boston butt, 10%; and joint effects plus rounding errors, —1%. If the objective is more precise evaluation of weight of lean cuts or primal cuts, improved techniques are especially needed for appraising belly and loin.

The cutting error variances for ham and Boston butt are similar, .090 and .084, respectively, in spite of the large difference in means for the two traits. Thus the error for Boston butt is relatively larger than that for ham. Coefficients of variation, computed as 100 times the ratio of standard

deviation of cutting errors to the mean, are given in table 2 so that relative cutting errors may be compared readily. Cutting errors are relatively large for Boston butt, loin, loin eye area, picnic and belly; intermediate for half carcass, primal cuts, lean cuts, and ham; and small for average backfat thickness and carcass length. In general, the coefficients of variation are largest for those traits produced by several distinct cutting steps. For example, Boston butt, loin, picnic and belly have relatively large errors, while carcass length and backfat thickness which are measured on opposite

TABLE 2. CUTTING VARIATION AND CORRELATION BETWEEN TRAIT DIFFERENCES AND CARCASS WEIGHT DIFFERENCES

Trait	Variance of cutting error	Coefficient of variation	Left-right repeatability	Percent increase in information from cutting both sides	Correlation between left-rt. difference for carcass wt. and left-rt. difference for trait
Half carcass, lb.	2.415	2.0	.72	16	—
Belly, lb.	.199	4.1	.76	14	−.13[a]
Loin, lb.	.346	5.3	.68	19	.47[b]
Ham, lb.	.090	2.3	.91	5	.21[b]
Picnic, lb.	.115	4.7	.59	26	.41[b]
B. butt, lb.	.084	6.4	.55	29	.22[b]
Lean cuts, lb.	.649	2.3	.87	7	.63[b]
Primal cuts, lb.	.819	1.9	.84	9	.57[b]
Length, cm.	.084	.4	.99	0.5	.04
Avg. backfat, cm.	.008	.2	.97	1.5	−.03
Loin eye area, tenth rib, sq. in.	.032	5.3	.85	8	−.03
Loin eye area, last rib, sq. in.	.027	4.7	.90	5	.001

[a] Statistically significant ($.01<P<.05$).
[b] Statistically significant ($P<.01$).

surfaces of a single cut have relatively small errors. The ham has relatively less cutting error than any of the other wholesale cuts.

Ability to discriminate between carcasses is a function of the variation among carcasses and the error variance. Variation among carcasses is, in turn, partly a function of variation in carcass proportions and partly a function of variation in carcass weight. The relative importance of cutting errors in obscuring differences among carcasses is inversely proportional to the repeatability (correlation) between left and right sides. Large cutting errors mask fewer real differences when variation among carcasses is large than when it is small. Thus, for example, the coefficient of variation for loin eye area was relatively large yet the observed repeatability (table 2) is high indicating that cutting errors do not seriously limit ability to discriminate between carcasses for this trait. If carcass weight varies, the

apparent variation among carcasses is increased, the observed repeatability is increased and cutting errors seem less important than if carcass weight were constant.

The additional information gained by cutting both sides decreases as repeatability increases since there is a greater tendency for both sides to yield the same information. If the variance per carcass is unity when only one side is cut, it is $\frac{1-r}{2}$ less than unity when both sides are cut. For example, the variance of a mean for picnic is reduced from 1 to .795 by averaging two sides. This corresponds to an increase of 26% in the information obtained per pig. Thus for measuring group differences, four pigs would yield approximately as much information as five would if only one side were cut. The increase in information for each trait is given in table 2. The increase is greatest for Boston butt, picnic and loin. Very little is gained by measuring length and backfat thickness on both sides.

The weight difference between carcass halves suggests that division of the carcass into unequal halves is responsible, at least in part, for the average left-right difference for various traits. Correlation coefficients were computed between left-right differences for weight of carcass halves and left-right differences for each of the other traits and are summarized in table 2. Failure to divide the carcass into equal halves is responsible for a significant part of the left-right differences for each lean cut. The heavier carcass side tends to yield heavier lean cuts but lighter belly. Removal of the head, opening of the belly (and lower shoulder), splitting of the back and bilateral asymmetry are possible sources of differences between sides. The average difference between sides is not significant for loin eye area nor do these data show significant correlation between left-right differences for loin eye area and left-right differences for carcass side. Thus bilateral asymmetry is not demonstrated and is assumed to be unimportant. It is doubtful that errors made in the removal of the head contribute much to differences for any of the traits since differences due to this source are probably minimized when the jowls are trimmed from the shoulders. Variation in the belly split is apt to cause weight differences between picnics while loins, Boston butts and hams more likely reflect variation in the back split. The relative effect of these sources of variation is not known but both are presumed important. Careful splitting of the carcass improves accuracy and precision when only one side is evaluated but nothing is gained when both sides are averaged.

Summary

The magnitude of cutting errors was determined for various carcass traits using 222 barrow carcasses. Traits investigated were weight of half carcass, belly, loin, picnic, Boston butt, skinned ham, four lean cuts, and

five primal cuts; length from front of first rib to aitch bone; average of four backfat measures; and loin eye area at tenth and last ribs.

The left side averaged heavier and yielded heavier loin, ham, picnic, lean cuts and primal cuts but lighter belly and Boston butt. Failure to divide the carcass into equal halves is partly responsible for these differences. No evidence of bilateral asymmetry was found. Biased values are obtained when either right or left side is consistently appraised, but not when both are averaged. Greater precision also results when both sides are averaged, especially for loin, picnic, Boston butt and belly. About one-half the cutting variation for lean cuts reflects lack of precision in cutting the loin. Ham weight is more reliably estimated than is any other wholesale cut or combination of cuts.

If only one side is evaluated it is important that it always be the same side. Careful splitting of the carcass is emphasized when only one side is cut.

Literature Cited

Kline, E. A. and Hazel, L. N. 1955. Loin area at tenth and last ribs as related to leanness of pork carcasses. J. Animal Sci. 14:659.

Report of the Pork Carcass Evaluation Committee, 1952. Cutting procedure, Proc. Fifth Ann. Reciprocal Meat Conf. 120.

BRUISED TISSUE. II. DETERMINATION OF THE AGE OF A BRUISE [1]

M. K. Hamdy, L. E. Kunkle and F. E. Deatherage [2]
The Ohio State University, Columbus, Ohio [3]

IF bruised tissue could be examined biochemically and/or grossly in such a manner so as to accurately establish the time lapse between wounding and examination, a means then would be available to assign the place of livestock damage and, in addition, might provide information as to cause. The increasing losses due to wounded and bruised livestock is of grave concern to producers and shippers of livestock as well as to the meat packing industry.

At the present time the determination of the age of a bruised tissue on a slaughtered animal is based on gross observations and color changes which are characteristic of a bruise. These changes have been found to be subject to variation (Hamdy *et al.*, 1956). The studies undertaken here were designed to devise quick and simple chemical and physico-chemical tests for the determination of the age of a bruise.

Material and Methods

Cattle and rabbits were used in this study. Bruise infliction and tissue sampling techniques were reported in a previous communication (Hamdy *et al.*, 1956).

The bilirubin albuminate solution was prepared as described by Shinowara, (1954).

Detection of bilirubin. The test for detecting bilirubin in a tissue was modified from that described by Naumann (1936). The bruised and control tissues were excised immediately after slaughtering the animals and immersed in a beaker containing Fouché's reagent.[4] The tissue samples were excised with 1.5 or more inches of normal tissue surrounding the bruised area and were scored to facilitate the penetration of the Fouché's reagent. After standing for 10–20 minutes at room temperature the tissue samples were examined. In the presence of bilirubin, the tissues will develop a blue to green color, depending on the concentration of this bile pigment. This blue-green color (probably biliverdin) is a reaction product of the oxidation of bilirubin by the ferric chloride in the trichloroacetic acid solution.

[1] Published with the approval of the Associate Director, The Ohio Agricultural Experiment Station. Journal Article No. 61?56.
[2] The authors gratefully acknowledge the financial support of the Farmer Cooperative Service, United States Department of Agriculture.
[3] Departments of Animal Science and Agricultural Biochemistry.
[4] Fouché's reagent is made by mixing 25 g. trichloroacetic acid, 10 ml. of 10% ferric chloride and 100 ml. distilled water.

Conductivity measurements. Specific conductance or conductivity is the reciprocal of the specific resistance and is expressed in reciprocal ohms. For the determination of the conductivity of a tissue a modified conductance cell was used. This cell consisted of two hypodermic needles (1 in., 18 gauge) which were filled with paraffin and employed as electrodes. These were mounted in a Lucite block (1.25 x 2.5 x 0.25) and separated by 1 inch. The resistance or conductance is measured by inserting the electrodes into the tissue, to the level of the Lucite block, and connecting the cell thus formed as one arm of a Wheatstone bridge. The Leeds and Northrup portable electric conductivity bridge No. 4960 was used. The cell was calibrated by measuring the resistance offered to a solution of known conductance (0.10 N KCl) according to the following equation from which the cell constant was calculated:

$$\text{Specific conductance} = \frac{\text{Cell constant}}{\text{Resistance}}$$

For measuring the conductivity of a tissue the needles of the cell were inserted in various parts of the tissue and approximately 10 readings, representing the resistance offered by the tissue, were obtained. The specific conductance was calculated by dividing the cell constant by the average resistance offered by the tissue.

It should be stated that the resistance of the control (non-bruised tissue) was made from an area symmetrically located to the bruised tissue.

Results and Discussion

Color test for the determination of the age of a bruise. This test is based on the experimental observation, previously reported (Hamdy *et al.*, 1956), that a bile pigment compound (bilirubin) was formed during the healing process of bruised tissue as a result of the degradation of the hemoglobin molecules present. This yellow pigment was found to reach a maximum concentration in bruised tissue after 4 days then decreased and finally disappeared upon complete healing.

Several animals were bruised, one bruise per animal, using approximately the same force. At various intervals during the healing process the animals were sacrificed. The bruised and control tissues were immediately excised, scored and tested for the presence of bilirubin. The bilirubin seemed to be bound to the protein molecules of the tissue confirming the results reported by Stenhager and Rideal (1936). The tissues were immersed in the Fouché's reagent for 10–20 minutes at room temperature and then examined. The results of these experiments are recorded in table 1 and indicate the absence of bilirubin in bruised tissue of less than 50–60 hours of age and in control (non-bruised) tissue. The color reaction of a bruised tissue was a diffused light blue at the age of 60–72 hours and diffused dark green when measured on the 4th and 5th days after infliction

of the bruise. This color reaction was most discernible at the periphery of the bruise and decreased in intensity towards the center, analogous to the yellow-green (orange) color usually observed on the skin over a bruised tissue. This seemed to be due to the hypermetabolic activities of the undamaged tissues at the periphery of the bruised area. After five and up to eight days this bile pigment was found to localize in high concentration in various areas of the bruised tissue most probably in macrophages. Therefore, the diffused color reaction was very light blue or absent and instead dark green areas or crystals (2–3 mm in diameter) were found when the tissue was allowed to remain longer (2–6 hours) in the Fouché's reagent, thus permitting more time for the reagent to diffuse and reach the high local concentration of bilirubin imbedded in the tissues. This bile pigment may undergo autoxidation when the atmospheric oxygen is sufficient and this test should be made directly upon sacrificing the animals.

TABLE 1. THE COLOR REACTION OF BRUISED TISSUES AT VARIOUS INTERVALS DURING THE HEALING PROCESS IN CATTLE

The age of the bruise	Tissue color after 10–20 minutes incubation in the Fouché's reagent at room temperature
Normal tissue	The tissue developed no color.
Zero-60 hours (fresh bruise)	The color of the bruised tissue was pink in the first few minutes and gradually turned brown. No blue color was detected.
60–72 hours (intermediate bruise)	The tissue developed a diffused very light blue color along with a dark brown color at 60 hrs. and blue at 72 hrs. This blue color could be clearly observed in the fat tissue.
3–5 days (slightly old bruise)	A diffused dark green color, especially at the periphery, along with a brown color (in the center) of the bruised tissues were detected.
5–8 days (old bruise)	This type of bruise developed slight or no blue color, but upon longer contact with the reagent,[a] dark green crystals were found imbedded in the bruised area of either the lean or the fat tissue.

[a] This color reaction was detected when the tissue was allowed to remain 2–6 hours in the Fouché's reagent.

Experiments on the hide of the bruised animals using this color test indicated its usefulness with the hide alone or in conjunction with bruised tissue for the determination of the age of the bruise. The same types of color reactions were obtained on the hide of the animals only in and around the areas where the bruises were inflicted, suggesting the possibility of biopsy experiments to determine the age of a bruised tissue in the living animal.

Specificity of the color reaction. Studies to investigate the specificity of this color test were conducted using biliverdin and other pigments which were established to be present in bruised tissue, such as hemoglobin, alkaline hematin and bilirubin (Hamdy *et al.*, 1956). Various concentrations of these pigments were adsorbed on a layer of talc in a Büchner funnel and tested for color development using Fouché's reagent (Naumann, 1936).

Bilirubin was found to be the only compound among the pigments tested which produced the blue and green color with Fouché's reagent. It was also found that the change of color from light blue to dark green is dependent on concentration of the adsorbed bilirubin; high concentration developed a dark green color. Alkaline hematin produced a pink color which immediately turned to brown while hemoglobin produced brown color. Biliverdin displayed no color with Fouché's reagent.

The sensitivity of this test to bilirubin. Naumann (1936) reported that the limit of detectability of bilirubin dissolved in weak NaOH, dilute alcohol and urine, freed from preformed bilirubin, were 0.006, 0.008 and 0.009 p.p.m. respectively. Since bilirubin appeared to be bound to the protein in the tissue, it was decided to investigate the limit of detectability of bilirubin albuminate solution (pH 7.4). The results revealed that the limit of detectability of bilirubin albuminate absorbed on a layer of talc was 0.01–0.008 p.p.m.

Further experiments were conducted using cattle tissues (lean and fat) for the adsorption of bilirubin albuminate. The tissues were cut to cubes 2 x 0.5 x 0.12 inches. One ml. containing various concentrations of bilirubin albuminate ranging from 1000 to 0.00001 p.p.m. was placed on each tissue sample. After four hours at room temperature in open atmosphere, or under vacuum for two hours, the adsorbed bilirubin was detected by adding 2 ml. of Fouché's reagent to the surface of the tissue. The results of these experiments showed that the limit of detectability of bilirubin albuminate adsorbed to the tissues was 50 to 100 parts per million.

It was also found that detection of the color was easier in fat tissues than in lean tissues and that adsorption of 50–100 p.p.m. bilirubin albuminate produced a light blue color which changed in intensity to reach a dark green color when the tissue adsorbed higher concentrations of bilirubin.

The effect of different tissues and species of animal on the color test. Tissues from various muscles of rabbits, lambs and cattle were tested with one ml. of bilirubin albuminate containing 100 p.p.m. of bilirubin as before. The results showed that the color test was not affected by location of muscle sample or animal specie.

The relation between conductivity measurements and the age of a bruise. During studies on bruises, iron, hemoglobin, bilirubin, nonprotein nitrogen and accumulation of fluid were found to be correlated with the age of a bruised tissue (Hamdy *et al.*, 1956). For example, hemoglobin and the accumulation of fluids were found to be higher in a bruised tissue than in a normal tissue, especially in the early stages of the bruise.

The accumulation of the fluid in a bruised tissue with its increased concentration of electrolytes was considered to be another aspect of the problem. It was recently reported by Merezhyhskii and Charkasava (1954) that the concentration of sodium increased in a bruised tissue, and then decreased to normal upon healing. They also stated that under conditions

of trauma cellular barriers were destroyed with respect to Na ions, resulting in an increased diffusion of sodium ions into the cells of the traumatized tissues and in a greater accumulation of Na^+ in the intercellular fluid.

Thus the conductivity of normal and bruised tissue in cattle was measured. The animals were bruised (one bruise per animal) and after 24 hours were slaughtered and the average resistances offered by the bruised tissues and by the control (non-bruised tissue) were obtained immediately after removing the hide. The control tissues were excised again from areas located symmetrically from the bruised tissue. The resistance offered by the tissues was measured and conductivities of the tissues were calculated using the cell constant, as previously described. This experiment was repeated several times and the mean conductivity value for a 24-hour-old

TABLE 2. THE RELATION BETWEEN THE CONDUCTIVITY AND THE AGE OF A BRUISED TISSUE IN CATTLE

Age of sample	No. of samples	Conductivity $\times 10^{-2}$ (ohm^{-1} cm^{-1})			
		Bruised	St. Dev.	Control [a]	St. Dev.
Just before killing	5	2.45	0.3	1.80	0.2
15 minutes	4	2.75	0.5	1.86	0.2
15 hours	6	4.30	0.6	1.65	0.3
24 hours	8	4.60	0.4	2.07	0.1
40 hours	7	5.40	0.3	2.05	0.1
3 days	6	4.00	0.3	1.95	0.2
4 days	6	2.95	0.3	2.00	0.1
7 days	5	1.95	0.2	1.90	0.2

[a] Control from a symmetrically located area as the bruised tissue sample.

bruise was found to be (4.6×10^{-2} ohm^{-1} cm^{-1}) ± 0.4 while the control sample was (2.07×10^{-2} ohm^{-1} cm^{-1}) ± 0.1 indicating a significant difference in conductivity between bruised and normal tissue.

To investigate the relationship between conductivity of bruised tissue and its age during the healing process, cattle were bruised using the same force as nearly as possible, and at different stages of healing were slaughtered and resistance measurements were made from which conductivity values were calculated (table 2). The conductivity of bruised tissue was affected by the age of the bruise; increasing during the early stages of healing to reach a maximum after 40 hours, and then decreasing to the normal value of the control upon complete healing. This suggests that the concentration of electrolytes increases in the first stages of healing and then subsequently descends to the normal value as healing is completed. Some variations were observed between the conductivity of different bruised tissues of the same age. This was found to be due to many factors, such as the size and the extent of the bruise and on the quantity of fat present in the tissue. When the conductivity of the bruised tissue and the

control were plotted against the age of the bruise, (figure 1) it was possible to approximate the age of an unknown bruise.

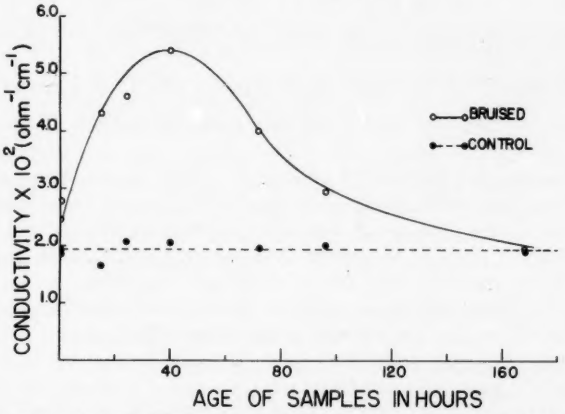

Figure 1. The relation between the conductivity and the age of a bruised tissue in cattle.

Summary

A simple color test was devised for approximating the age of a bruise. This test is based on the presence or absence of bilirubin in a bruised tissue.

Conductivity measurements also can be used for determining the age of a bruise. It was found that the resistance offered by bruised tissue is quantitatively affected by the age of the bruise.

Using both the color test and the conductivity measurements of a bruised and control tissue, excised from symmetrically located areas, it is possible to determine the approximate age of a bruise.

Literature Cited

Hamdy, M. K., G. Y. Shinowara and F. E. Deatherage. 1956. Bruised tissue. I. Some biochemical changes occurring during healing. (In press.)

Hamdy, M. K., F. E. Deatherage, L. E. Kunkle and M. S. Rheins. 1956a. III. Some factors affecting experimental bruises. (In press.)

Naumann, H. N. CIX Studies on bile pigments. 1936. II. A new test for bilirubin in the urine and its use for detection of bilirubin in normal urine. Biochem. J. 30:762.

Merezhynskii, M. F. and L. S. Charkasava. 1954. Participation of certain electrolytes in the overall metabolic reaction of the organism against tumors. Vesti Akad. Navuk Belarus S.S.R. No. 2:95.

Shinowara, G. 1954. Spectrophotometric studies on blood serum and plasma. Am. J. Clin. Path. 24:696.

Stenhagen, E. and E. K. Rideal. CXCV. 1939. The interaction between porphyrins and lipoid and protein monalayers. Biochem. J. 33:1591.

BRUISED TISSUE

III. SOME FACTORS AFFECTING EXPERIMENTAL BRUISES [1]

M. K. Hamdy, L. E. Kunkle, M. S. Rheins and F. E. Deatherage [2]

The Ohio State University, Columbus, Ohio [3]

IN a previous investigation (Hamdy *et al.*, 1956) it was shown that the time required for the gross changes to occur in bruised tissue is variable. The extent of the damage of similarly administered bruises also varied with individual animals. In general, however, the sequence of the visible changes which occurred was always consistent, regardless of the animal species being tested, the force applied or the location of the bruise. For example, the blue-green color of the bruise invariably appeared before the green-yellow (orange) color. The latter was never observed in a bruise prior to 60–72 hours, and was found to be associated with the bile pigment, bilirubin (Hamdy *et al.*, 1956). Lemberg and Wyndham (1936) reported that biliverdin is reduced to bilirubin rapidly enough in animal tissues, particularly in the liver, spleen, kidney, and brain to suggest that biliverdin is the primary product of hemoglobin degradation and that bilirubin results from the reduction of biliverdin in the body.

Studies were undertaken to examine some of the factors which may affect the experimental bruising of animals. This information might be of value in ascertaining the age of an unknown bruise.

Material and Methods

Cattle and rabbits were used in these studies. Methods for inflicting bruises and for recovery and preparation of tissue for examination were reported previously (Hamdy *et al.*, 1956).

Bilirubin formation and detection as a criterion for factors affecting bruises. By employing the color test (Hamdy *et al.*, 1956) for the detection of bilirubin in a bruised tissue, it was found that the minimum measurable concentration of this bile pigment in a tissue was 50–100 p.p.m. Therefore, examination of bruised tissue of less than 50–60 hours and sometimes 72 hours of age, failed to reveal the presence of bilirubin as measured by the color test (blue color). This may be due to the slow degradation of hemoglobin and the subsequent insufficient production of bilirubin in the bruised tissue during the early stages of healing.

[1] Published with the approval of the Associate Director, The Ohio Agricultural Experiment Station. Journal Article No. 64–56.

[2] The authors gratefully acknowledge the financial support of the Farmer Cooperative Service, United States Department of Agriculture.

[3] Departments of Animal Science, Agricultural Biochemistry and Bacteriology.

Results

Influence of previous bruising on rate of healing. Young adult rabbits six months of age were divided into groups. Each animal of the first group was bruised and sacrificed at a stated interval. The tissues were examined grossly and by the Fouché's reagent for bilirubin (Hamdy *et al.*, 1956). Another group of animals was treated similarly, but each member was bruised a second time three days later. A third group received three bruises, the last bruise inflicted two days after the second. It should be stated that the same force was used to inflict the bruises on the animals. All animals were examined grossly and the tissues by the Fouché's reagent as before. The results recorded in table 1 indicate that the time required for complete healing as determined by gross observation and by the detection of bilirubin is shorter for each subsequent bruise. The second bruise (animals

TABLE 1. HEALING TIME AND BILIRUBIN DETECTION IN RABBITS RECEIVING MULTIPLE BRUISES

No. of animals	Healing time, days		Bilirubin detection, hr.	
	Mean	St. Dev.	Mean	St. Dev.
		First Bruise		
40	7.9	0.3	70	1.4
		Second Bruise		
40	6.6	0.2	56	1.5
		Third Bruise		
40	5.9	0.1	46	1.8

which had been bruised twice) healed in an average of 6.6 days as compared to 7.9 days for those rabbits which had been bruised only once. The last bruise of animals which had been bruised three times healed in an average time of 5.9 days, i.e., faster than did either the first or second bruises.

Similarly, bilirubin was detected earlier in the tissues following each subsequent bruise (70 hours, single bruise; 56 hours, second bruise; 46 hours, third bruise).

Attempts to passively transfer the healing factor. In view of these results an experiment was conducted to investigate the possible transferability of the "healing factor" or factors in the blood of a bruised animal.

A rabbit was bruised weekly for one month. Five days following the last bruising, 20.0 ml. of blood were withdrawn by cardiac puncture into 3.0 ml. of anticoagulant.[4] This blood was injected intravenously into a

[4] A.C.D. solution. Each 100 ml. contains 2.45 gm. dextrose, 2.20 gm. sodium citrate and 0.80 gm. citric acid.

normal animal which was bruised 24 hours after the transfusion. Animals treated in the following manner served as controls: (1) Bruised rabbit which had not received a blood transfusion, (2) Bruised rabbit previously injected with a 3.0 ml. of the anticoagulant, and (3) Bruised rabbit previously injected intravenously with 20.0 ml. of normal blood from a non-bruised rabbit.

Several other groups of animals were treated similarly. At various intervals the test groups were sacrificed, the bruised tissues examined grossly and tested for bilirubin as before.

The results recorded in table 2 demonstrate the accelerated healing of animals transfused with blood from bruised animals. Complete healing of bruised tissues in the transfused rabbits, as determined grossly, occurred in an average of 6.1 days as compared to 7.9 days for the control animals.

TABLE 2. PASSIVE TRANSFER OF HEALING FACTOR

No. of animals bruised	Source of blood transfused 20.0 ml.	Healing time (days)		Bilirubin detection (hours)	
		Mean	St. Dev.	Mean	St. Dev.
5	Bruised rabbits	6.1	0.3	43	1.6
14	No treatment (control)	7.9	0.3	70	1.4
4	Normal rabbits	7.8	0.2	72	1.4
3	No treatment other than injection of 3.0 ml. of anticoagulant [a]	8.0	0.3	71	1.5

[a] A.C.D. Solution. Each 100 ml. contains: 2.45 gm. dextrose, 2.20 gm. sodium citrate and 0.80 gm citric acid.

The presence of bilirubin also was detected earlier in the test animals (43 hours as compared with 70 hours).

Rate of healing in different animal species. Cattle, hogs, sheep and rabbits were bruised and the damaged areas were examined grossly before and after slaughter. Tissue samples also were tested for bilirubin formation. In general, the rate at which the gross changes associated with bruising and healing occurred was approximately the same, regardless of the species being studied. The presence of bilirubin in the damaged tissues of the various animals was first observed between 60 and 72 hours from the time of bruising. It should be stated, however, that some differences were noted between individuals of the same species.

Influence of age on rate of healing. Eight young female rabbits (5–8 months of age) were bruised once over the gluteus muscle with approximately the same force. Two rabbits from each age group were sacrificed after 55 hours, 70 hours, 3 days and seven days from the time of bruising. The bruised tissues of animals sacrificed at the latter two intervals were

excised and weighed. Bilirubin was detected by the Fouché's reagent in the tissue of rabbits sacrificed after 55 and 70 hours. Although the number of animals used in this experiment was not large, it can be stated that the bruises inflicted on younger rabbits (2–5 months of age) appeared to heal significantly more rapidly than did bruises on adult rabbits (5–8 months of age). Also the quantity of damaged tissue in the older animals was observed to be greater than that found in the younger rabbits. Bilirubin was detected at 55 hours in the younger but not in the older animals, indicating acceleration of hemoglobin degradation (a measure of healing).

Relationship of force applied to extent of bruising. Both cattle and rabbits were bruised by various techniques. The cattle were struck with hammers of different masses applied with varying velocities, rubber pressure tubing and wooden sticks, while the rabbits were contused with the rubber pressure tubing with changes in the velocities and the number of blows administered. All bruises were inflicted in the same area of each animal species regardless of the force of bruising, e.g. the biceps muscles in the case of cattle and the gluteus muscles in rabbits. It should be mentioned that only one bruise was inflicted on each of the animals in order to avoid the stimulatory effect of bruising on the rate of healing of subsequent bruises. These experiments were repeated several times and at various intervals during the healing process, animals were sacrificed and the bruised tissues were examined and compared.

The results of these experiments indicated that the extent and the severity of the damaged tissues was affected directly by both the mass and the velocity of the wounding instrument employed to inflict the bruise. An instrument with a large mass and a neglible velocity produced minimal or no bruising, while another instrument with a small mass and a large velocity, resulted in a small, discrete but severe type of bruise. When both the mass and the velocity are increased, the area and depth of the bruised tissue also are increased. This in turn lead to a prolongation of the time necessary for complete healing when compared to the time required for the superficial type of bruise. Regardless of the force employed to inflict the bruise, the sequence of visible and chemical changes associated with healing were the same.

Location of the bruise. Cattle and rabbits were used in this experiment. Each cow was bruised with approximately the same force, using a 7 lb. hammer which traveled a distance of about three feet in approximately one second. Ten blows from a rubber pressure tubing one-half inch thick, traveling two feet in about one-half second were used for bruising rabbits. Various areas of the animals were bruised (a single bruise per animal). The bruised tissues were examined grossly before and after slaughtering.

The bruises inflicted over the gluteus muscles, the triceps, the biceps and the trapezius muscles were deeper than were those inflicted over the lubo-dorsal fascia and the serratus muscles. Bruises over the latter muscles tended to spread laterally. More tissue damage with accompanying hemor-

rhagic edema, extending into the intermuscular tissues, was observed when the bruises were inflicted close to the tuber coxae, tuber ischii, the scapula and the patella.

Influence of Blood Pressure on Bruising. Cattle were bruised with approximately the same force before and after bleeding. The bruised tissues were examined by the usual procedures.

The results bore out the contention that bruises can be produced in the animals before and after stunning and before but not after bleeding, i.e., when the blood pressure approaches zero.

Discussion

The above data emphasize the complexity of factors associated with the phenomenon of bruising. Not only is the force exerted to inflict the bruise important to the extent of tissue damage, but also the particular tissue or tissues involved. The thickness or density of the tissue and its vascularity undoubtedly influence the degree of bruising. Since animals within the same species varied as to their susceptibility to bruising, it seemed likely that differences in vascular fragility, age of the animal, and effects of previous bruising could, at least in part, be responsible for these observations. Although changes or variation in vascular fragility were considered, this aspect was not deliberately included in the present study. However, the increased rate of healing observed in the younger rabbits as compared with that seen with older animals suggests differences in metabolic activity which probably are reflected in the vasculature. The enhanced healing seen in the younger animals supports the finding of Howes and Harvey (1932) who concluded that this enhancement may be associated with the fact that fibroplasia occurs earlier and is less retarded in younger animals.

Of particular interest were the results obtained regarding the increased healing rate of rabbits which had been repeatedly bruised. Similar results were observed in rabbits bruised subsequent to receiving a transfusion of whole blood from previously bruised animals. Apparently the same phenomena operate in these instances as have been reported by other workers in studies of wound healing (Young *et al.*, 1941; Loofbourow *et al.*, 1940; Cook *et al.*, 1942; Engley *et al.*, 1955 and Sandblom, 1949). At least in one such study (Engley *et al.*, 1955), it was possible to passively transfer the accelerated healing factors by means of serum; in the study reported here whole blood only was transfused. No single substance or factor has been isolated which can account for the accelerated healing of a secondary wound. Young *et al.*, (1941) have reported the presence of growth promoting, hormone-like substances during healing, which apparently contribute to healing enhancement. Although no indisputable evidence has been presented for an isoimmune mechanism, the possibility still exists that antibody may play a role in the healing of second wounds. Numerous studies concerned with skin graft rejections have strongly suggested an isoim-

munizing mechanism as being responsible for the tissue destruction, yet, here too, the demonstration of antibody by serologic techniques has thus far met with failure.

Summary

Regardless of the animal specie tested or the site of the bruise, the sequence of the visible and the measurable chemical changes associated with bruise healing were consistent. This is in agreement with earlier observations.

The results of studying comparative healing rates between rabbits of different ages bore out similar findings of other investigators inasmuch as the younger animals healed significantly more rapidly.

Enhanced healing was observed in animals which had been bruised previously. Each of three successive bruises healed more rapidly than did the preceding one.

The factor or factors responsible for this accelerated healing could at least in part be passively transferred in the whole blood from a previously bruised animal.

Literature Cited

Engley, F. B., Jr., M. Allgower and C. D. Snyder. 1955. The role of wound healing in relation to the tissue and humoral responses in homotransplantation. Ann. New York Acad. Sci. 59:326.

Cook, E. S. and J. C. Fardon. 1942. The wound hormone concept in wound healing. Surgery, Gynec. & Obst. 75:220.

Hamdy, M. K., G. Y. Shinowara and F. E. Deatherage. 1956. Bruise tissue I. Some biochemical changes occurring during healing. (In press.)

Hamdy, M. K., L. E. Kunkle and F. E. Deatherage. 1956. Bruise tissue II. Determination of the age of a bruise. (In press.)

Howes, E. L. and S. C. Harvey. 1932. The age factor in the velocity of the growth of fibroblasts in the healing wound. J. Expt. Med. 55:577.

Lemberg, R., and R. A. Wyndham. 1936. CLXV. Reduction of biliverdin to bilirubin in tissues. Biochem. J. 30:1147.

Loofbourow, J. R., C. M. Dwyer and M. M. Lane. 1940. Proliferation-promoting intercellular hormones. I. Quantitative studies of factors produced by injured animal tissue cells. Biochem. J. 34:432.

Merezhynskii, M. F., E. S. Yadvinskaya and V. S. Ivanova. 1954. Changes in the ascorbic acid level of the blood and tissues in skin burns. Ukrain. Biokhim. Zhur. 26:435 (in Russian).

Sandblom, P. 1949. The effect of injury on wound healing. Ann. Surg. 129:305.

Tier, H., A. Kiljunen and T. Putkonen. 1951. Existence of a growth-promoting factor in the skin of the white rat. Ann. Chir. Gynaec. Feen. 40:61.

Young, J. S., J. A. Young and M. Young. 1941. Some observations on the healing of experimental wounds in the skin of the rabbit. J. Path. Bact. 52:225.

ISOLATION AND IDENTIFICATION OF COMPOUNDS FROM AUTOLYZED YEAST, ALFALFA MEAL, AND CASEIN HYDROLYSATE WITH CELLULOLYTIC FACTOR ACTIVITY FOR RUMEN MICROORGANISMS *IN VITRO*[1, 2]

BURK A. DEHORITY, ORVILLE G. BENTLEY, RONALD R. JOHNSON AND A. L. MOXON

Department of Animal Science, Ohio Agr. Exp. Sta., Wooster, and Department of Agricultural Biochemistry, The Ohio State University, Columbus

DURING the development of *in vitro* techniques for the study of cellulose digestion by rumen bacteria, several investigators have found a requirement for unidentified cellulolytic factors, which are present in a large number of natural materials. Rumen fluid is an excellent source of these cellulolytic factors and Doetsch *et al.* (1952) and McNeill *et al.* (1954) have further reported that it contains unknown factors essential for optimal growth of the total rumen population. Burroughs *et al.* (1950; 1950a; 1950b), Ruf *et al.* (1953) and Bentley *et al.* (1954) have demonstrated cellulolytic factor activity in hot water extracts of alfalfa leaf meal, timothy hay, ladino clover and cow manure, and also in autolyzed yeast, dried brewers yeast, distillers dried fermentation solubles and molasses. Partial hydrolysates (acid, alkali and enzymatic) of casein, soybean protein, chicken feathers, hair, and blood meal were also found to stimulate cellulose digestion (Hall *et al.*, 1954).

Fractionation studies on rumen juice indicated that the cellulolytic factor activity was contained in the volatile fatty acid fraction, which led to the observation that valeric, caproic, iso-butyric and iso-valeric acids stimulated cellulose digestion, whereas acetic, propionic and butyric acids did not (Bentley *et al.*, 1954a; 1955). Analysis of the volatile fatty acid fraction from rumen juice indicated that of these active acids, n-valeric acid was the major constituent. The importance of these acids in rumen bacterial nutrition was further substantiated by Bryant and Doetsch (1955) who found that *Bacteroides succinogenes,* isolated from rumen contents, required a combination of a branched chain fatty acid (iso-butyric, iso-valeric or DL-α-methyl-n-butyric acid) and a straight chain acid (valeric or caproic acid) for growth on a purified medium.

On the other hand, Bentley *et al.* (1955) found that the cellulolytic factor activity of alfalfa meal, yeast extract and dried distillers solubles

[1] Approved for publication as a journal article, No. 73–56 by the Associate Director of the Ohio Agricultural Experiment Station. A report of this work was presented at the annual meeting of the American Society of Animal Production, November 23–24, 1956; and an abstract is published in J. Animal Sci. 15:1257, 1956.

[2] The studies reported herein were supported in part by a Grant-in-Aid from Swift and Company, Chicago, Illinois.

could not be steam distilled from acid solution. Hall. *et al.* (1955) also found this to be true with the activity of partial hydrolysates of casein, feather meal and gelatin. These findings suggested that the cellulolytic factor activity contained in these natural materials was not one of the volatile short chained fatty acids. From their work on partial hydrolysates of certain proteins Hall *et al.* (1954) have postulated that the cellulolytic factor(s) may be a water soluble peptide. In support of this theory is the observation that certain amino acids, particularly valine and proline, have been found to stimulate cellulose digestion (Bentley *et al.*, 1954a; 1955; Hershberger, 1955).

The isolation and identification of the cellulolytic factors of autolyzed yeast, alfalfa meal and casein hydrolysate was thus initiated by investigating that fraction containing the amino acids and water soluble peptides.

Experimental

In Vitro Rumen Fermentation Technique

The *in vitro* rumen fermentation technique used in this work was essentially the same as previously reported from this laboratory (Bentley *et al.*, 1955). One change made in the basal medium was to decrease the urea concentration from 0.168 gm. to 0.126 gm. per 100 ml. An improvement in the procedure for collection of the rumen microflora was made by drawing a line one inch from the bottom of the celluloid liner placed inside the Sharples supercentrifuge. Only the sediment above this line was used for innoculation of the *in vitro* fermentation flasks.

Fractionation Procedures

Dowex—50 ion exchange resin. Hot water extracts of alfalfa meal and aqueous solutions of autolyzed yeast were initially fractionated by passage through a bed of Dowex–50 ion exchange resin. The Dowex–50 was placed in a Buchner funnel and washed with a 25% NaOH solution, using suction, to remove any alkali displacable impurities. The resin was then converted to the H+ form by washing with 4N HCl until the filtrate was distinctly acid. Next, the resin was washed repeatedly with distilled water until the filtrate became neutral, thus signifying that all of the excess acid had been removed. The resin was then ready for use.

The extract was poured onto the resin bed, and the bed washed repeatedly with distilled water to flush any material not adsorbed by the resin into the filtrate. This solution shall be referred to as the Dowex–50 filtrate (D-50 filtrate) in the following sections. The D-50 filtrate was found to contain only a minute trace of ninhydrin positive material. The resin bed was then washed with a 50% NH_4OH solution until all of the ninhydrin positive material had been eluted. This was determined by spotting successive portions of the eluate on filter paper, drying and spraying with

ninhydrin. Removal of ammonia from this eluate was accomplished by boiling. This eluate will be called the Dowex–50 50% NH_4OH eluate (D-50 eluate).

Charcoal (Darco G-60) treatment. The Dowex–50 50% NH_4OH eluate was purified further by passage through a charcoal (Darco G-60) bed in a Buchner funnel. The charcoal bed was then washed with distilled water until the filtrate was free of ninhydrin positive material. A large volume of distilled water in relation to the sample volume was required for this procedure. This filtrate will be referred to as the charcoal filtrate of Dowex–50 50% NH_4OH eluate (C.F. of D-50 eluate).

Preparative scale paper chromatography. The C.F. of D-50 eluate was further fractionated by large scale unidimensional ascending paper chromatography. A large wooden balance cabinet (36″ high × 36″ wide × 18″ deep) with glass panels on the front, top and both sides, was used for a chromatographic chamber. The entire front panel of the cabinet slides upward allowing easy access to the chamber and the grooves are backed by felt to decrease evaporation. The wooden surfaces exposed on the interior of the cabinet were covered with paraffin, to decrease absorbtion of the solvents. Glass rods served as supports for the paper chromatograms. Whatman No. 1 filter paper sheets (13″ × 22½″) were used for chromatographic separation of the C.F. of D-50 eluate which was applied as a streak along the base line. The solvent system utilized for this separation was 80% phenol-20% water, and was placed in a large porcelain pan at the bottom of the chamber. The chromatograms were developed for 36 to 48 hours at room temperature.

Elution of the chromatograms. The developed chromatograms were removed from the chamber, air dried overnight in a hood, and then sprayed with a large excess of ether to remove any residual phenol or phenol decomposition products. After allowing the ether to evaporate, the chromatograms were dried in an oven at 105° for 15 minutes. Upon removal of the paper sheets from the oven, the separated amino acids were visible as flourescent zones under ultraviolet light. It was found that a non-flourescent zone consistently appeared approximately between the R_f values 0.60 and 0.65. The chromatograms were divided at this line into two portions, and the material from each portion was eluted with hot water. The solution obtained from that portion of the chromatogram between the non-flouresc-ing zone and the solvent front was termed the top portion of the phenol chromatogram (T.P.P.C.), while the eluate from the zone to the base line was called the bottom portion of the phenol chromatogram (B.P.P.C.). These solutions were concentrated by boiling before being tested in the artificial rumen.

Chromatographic identification of amino acids. The amino acids from those fractions showing cellulolytic factor activity were identified by unidimensional ascending paper chromatography. A number of the well known

amino acid solvents were used for this purpose (Block *et al.*, 1955). Repeatable results in three to four solvents constituted identification.

Results

Autolyzed Yeast (Difco yeast extract). The results of the preliminary fractionation of yeast extract (Dowex–50 and charcoal treatment) are

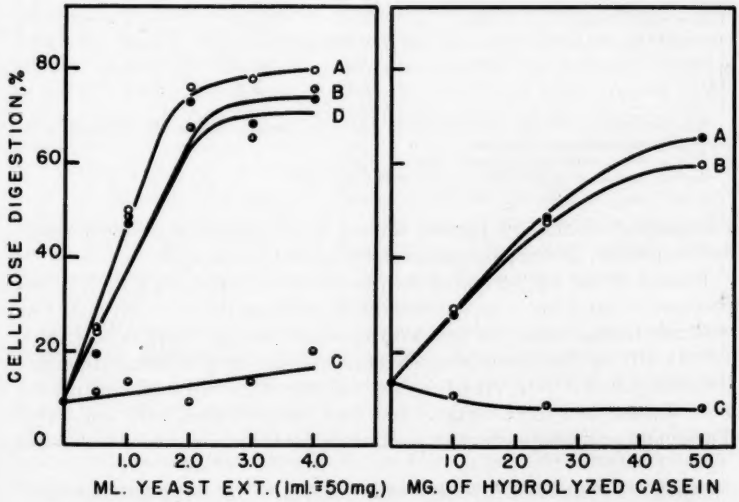

Figure 1 (Left). The cellulolytic factor activity *in vitro* of yeast extract, and the fractions prepared from this extract. (A) Yeast extract; (B) Dowex—50 50% NH_4OH eluate of yeast extract; (C) Dowex—50 filtrate of yeast extract; (D) Charcoal filtrate of the Dowex—50 50% NH_4OH eluate of yeast extract.

Figure 2 (Right). The cellulolytic factor activity *in vitro* of hydrolyzed casein and the fractions of hydrolyzed casein prepared by large scale paper chromatography. (A) Casein hydrolysate; (B) T.P.P.C. (top portion of phenol chromatogram) of casein hydrolysate; (C) B.P.P.C. (bottom portion of phenol chromatogram) of casein hydrolysate.

summarized in figure 1. These curves constitute the average results obtained from two *in vitro* rumen fermentations. It is clear that the cellulolytic factor activity of yeast extract is present in the Dowex–50 50% NH_4OH eluate, and the charcoal filtrate of this eluate. The cellulolytic factor activities of the fractions of C.F. of D–50 eluate obtained by paper chromatography, using 80% phenol as a solvent, are shown in table 1. These results indicate that the major portion of the cellulolytic factor activity was found in the top portion of the phenol chromatogram (above R_f 0.65). Paper chro-

TABLE 1. CELLULOLYTIC FACTOR ACTIVITY OF THE C.F. OF D-50 ELUATE[a] OF AUTOLYZED YEAST AFTER SEPARATION BY PAPER CHROMATOGRAPHY WITH 80% PHENOL

Additions to basal medium	Cellulose digestion, %				
	Exp. 1	Exp. 2	Exp. 3		
	3/12/56	4/3/56	5/1/56	5/8/56	
None	7.7	24.7	8.8	10.0	13.4
Valeric acid, 30 mg.	61.0	59.8	74.6	62.1	54.2
C.F. of D-50 eluate[a]	....	61.0	....	58.7	42.9
T.P.P.C.[b]	65.5	63.2	63.2	46.2	58.7
B.P.P.C.[c]	12.8	31.5	8.8	14.5	....

[a] Charcoal filtrate of Dowex–50 50% NH_4OH eluate. The quantity utilized was equivalent to the amount of chromatographic eluate used.
[b] Top portion of phenol chromatogram (above R_f 0.65).
[c] Bottom portion of phenol chromatogram (below R_f 0.65).

matography of this top portion showed it to contain five animo acids; valine, proline, leucine, isoleucine and γ-aminobutyric acid.

Enough of this top portion of the phenol chromatograms (T.P.P.C.) was prepared to carry out a second separation, utilizing different solvents. Two separations were made, the first with pyridine: tertiary amyl alcohol: H_2O (35:35:30) as the developing solvent, and the second using secondary butyl alcohol: 3% NH_4OH (3:1). After development, these chromatograms were divided into three sections and each section eluted with hot water. The amino acid composition of the three eluates was determined by paper

TABLE 2. CELLULOLYTIC FACTOR ACTIVITY OF THE SEPARATED COMPONENTS FROM T.P.P.C.[a] OF AUTOLYZED YEAST

Additions to basal medium	Cellulose Digestion, %			
	4/16/56		5/3/56	
None		12.2		4.3
Valeric acid, 30 mg.	Controls	61.0	Controls	40.6
C. F. of D-50 eluate[b]		37.2		62.0
T.P.P.C.		39.4		19.0
Fraction I[c]		29.2		34.9
Fraction II[d]	Rechromatographing	33.8	Rechromatographing	49.6
Fraction III[e]	Solvent-pyridine:	8.8	Solvent-sec-butyl	14.5
Fraction I plus II	tert-amyl alcohol:		alcohol: 3% NH_4OH	45.1
Fraction I plus III	H_2O			39.4
Fraction II plus III		28.1		31.5

[a] Top portion of phenol chromatogram (above R_f 0.65).
[b] Charcoal filtrate of Dowex–50 50% NH_4OH eluate. The quantity utilized was equivalent to the amount of chromatographic eluate used.
[c] Leucines.
[d] Valine and proline.
[e] γ-aminobutyric acid.
} Identified by paper chromatography.

chromatography, and similar identifications were obtained from both separations. Fraction I contained the fastest moving component, which was found to be the leucines (leucine and isoleucine). Fraction II contained the middle two amino acids which were valine and proline, while fraction III was γ-aminobutyric acid. The fractions thus obtained were then tested for factor activity in the *in vitro* rumen fermentation, and the results are shown in table 2.

The T.P.P.C. used for the separation by pyridine: tert.-amyl alcohol: H_2O was the same one used in experiment 2, table 1. Separation by sec.-butyl alcohol 3% NH_4OH was made using the same T.P.P.C. as reported in experiment 3 of table 1. No explanation for the low cellulose digestion of the T.P.P.C. in this second experiment (5/3/56) of table 2 can be made, but the data presented in table 1 and the activity of the separated components of this solution, clearly indicate its activity. Occasionally this lack of cellulose digestion for no apparent reason, is observed.

It can be concluded from the results presented in table 2 that the cellulolytic factor activity of T.P.P.C., and thus of yeast extract, lies in the amino acids valine, proline and the leucines. Experiments utilizing these known amino acids as cellulolytic factors in the *in vitro* rumen fermentation are presented at the end of this section.

Casein Hydrolysate. Commercial casein hydrolysate (acid) obtained from Nutritional Biochemicals Corporation was used as a cellulolytic factor source for these experiments. Because of the relative purity of this material, it was felt that preliminary purification with Dowex–50 ion exchange resin and charcoal, as used with yeast extract, was not necessary. The casein hydrolysate was applied directly to the filter paper sheets and chromatograped in 80% phenol. The chromatograms were divided into two sections, at approximately R_f 0.65, and the two sections were eluted with hot water. These eluates (T.P.P.C. and B.P.P.C.) were then passed through charcoal to remove any phenolic materials which may be toxic to rumen microorganisms. As before, the T.P.P.C. and B.P.P.C. were tested for factor activity *in vitro,* and the results are shown in figure 2. The curves shown are a summary of two experiments using the same T.P.P.C., and the results are in agreement with an experiment utilizing T.P.P.C. prepared at a different time. The cellulolytic factor activity of casein hydrolysate was found to be entirely in the T.P.P.C., while the B.P.P.C. was found to inhibit cellulose digestion slightly. Paper chromatographic analysis of the T.P.P.C. of casein hydrolysate revealed the amino acids valine, proline, the leucines and trace amounts of an unknown amino acid. From available R_f values in a number of solvents, it is believed that this unknown amino acid may be methionine sulfoxide.

An inhibition of cellulose digestion was observed when levels of casein hydrolysate exceeding 100–150 mg. per flask were used. Whether this inhibition is caused by an excess of certain amino acids, or simply by an excess of all amino acids is not known.

Alfalfa Extract. Hot water extracts of alfalfa meal were prepared by mixing the ground alfalfa with ten parts of water and bringing the mixture to a boil. The mixture was then filtered through a Buchner funnel, and this filtrate constituted the alfalfa extract. This extract was subjected to the Dowex–50 resin and charcoal treatments. Figure 3 illustrates the cellulolytic factor activity of these various fractions. These response curves are

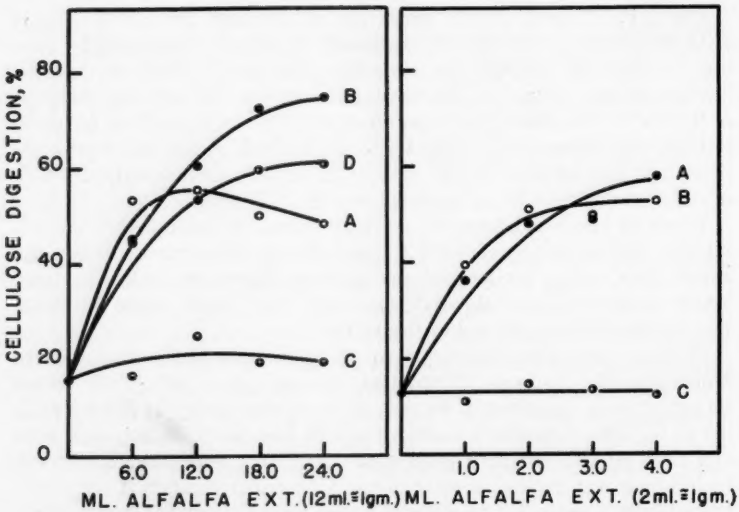

Figure 3 (Left). The cellulolytic factor activity *in vitro* of alfalfa extract, and the prepared fractions from this extract. (A) Crude alfalfa extract; (B) Dowex—50 50% NH_4OH eluate of alfalfa extract; (C) Dowex—50 filtrate of alfalfa extract; (D) Charcoal filtrate of Dowex—50 50% NH_4OH eluate of yeast extract.

Figure 4 (Right). The cellulolytic factor activity *in vitro* of the charcoal filtrate of Dowex—50 50% NH_4OH eluate of alfalfa extract and the fractions of this filtrate prepared by large scale paper chromatography. (A) Charcoal filtrate of Dowex—50 50% NH_4OH eluate of alfalra extract; (B) T.P.P.C. (top portion of phenol chromatogram); (C) B.P.P.C. (bottom portion of phenol chromatogram).

based upon data obtained in two separate *in vitro* rumen fermentations. Similar results were obtained from another alfalfa extract prepared in the same manner. The T.P.P.C. and B.P.P.C. of the C.F. of D–50 eluate were prepared as previously described, and their cellulolytic factor activity determined. These results are shown in figure 4, and represent the average of two determinations. As was found with yeast extract and casein hydrolysate the activity occurred in the T.P.P.C. Paper chromatography of this solution revealed the presence of the amino acids valine, proline, the leucines and γ-aminobutyric acid.

It will be noted in figure 3 that passage through charcoal of the D-50 eluate resulted in the loss of a portion of the cellulolytic factor activity. Paper chromatograms of these solutions indicated that charcoal treatment of the D–50 eluate decreased the content of the leucines. This possibility was investigated by comparing the ratio of the leucines to valine as determined on paper chromatograms with a densitometer. The ratio of the leucines to valine in alfalfa extract was approximately 1:0; in D–50 eluate 0.88; and in C.F. of D–50 eluate 0.21. Apparently the leucines were partially adsorbed by the charcoal, and not eluted with distilled water.

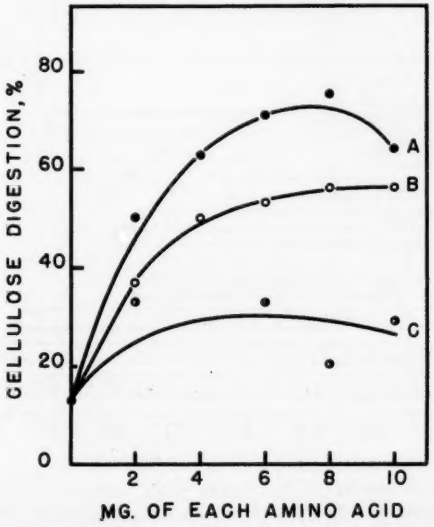

Figure 5. The cellulolytic factor activity *in vitro* of the amino acids valine and proline. (A) Valine plus proline; (B) Valine; (C) Proline.

Known Amino Acids. Those amino acids which were found to be consistently present in the T.P.P.C. of the materials studied were tested in the *in vitro* rumen fermentation for cellulolytic factor activity. Response curves, using various levels of the amino acids, were run to determine the level at which maximum cellulose digestion was obtained. Figure 5 illustrates a typical response curve with valine, proline and valine plus proline. From this and similar experiments it was concluded that 6 mg. would be the optimum level for testing the amino acids.

Figure 6 shows the cellulolytic factor activity of the various amino acids singly and in combination with each other. The data shown in figure 6, which are an average of two experiments, confirm the results previously reported from our laboratory on the cellulolytic factor activity of certain

amino acids (Bentley *et al.*, 1954a; Hershberger, (1955). As can be seen in figure 6, γ-aminobutyric acid possesses no cellulolytic factor activity, while proline exhibits activity. Also, γ-aminobutyric acid did not show an additive effect when tested in combination with the other amino acids. Valine and the leucines (half leucine and half isoleucine) show a somewhat greater stimulation for cellulose digestion than proline. When leucine and isoleucine were tested singly for cellulolytic factor activity, leucine was found to show the greatest stimulation; but since both of these amino acids were found in the materials examined, a mixture was employed for these studies. It is thought that valine and the leucines may function in the same manner as celluloytic factors, since a mixture of the two did not

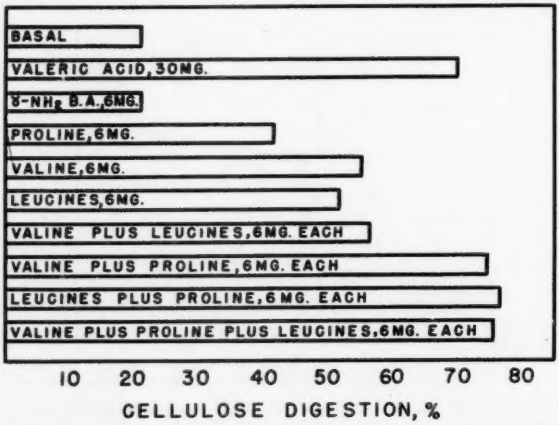

Figure 6. Additive effects of the amino acids valine, proline, the leucines, and γ—aminobutyric acid (γ—NH_2—B.A.) as cellulolytic factor *in vitro*.

show an increase in cellulose digestion. When proline was added with either valine or the leucines an additive effect was observed. This particular effect is more apparent in figure 5. A mixture of valine, proline and the leucines gave no further stimulation, again indicating that the function of valine and the leucines may be similar.

At this point the question arose as to whether an additive effect of these various cellulolytic factors, at their optimum concentrations, might be masked by a limiting cellulose substrate. An investigation of the effect of substrate upon cellulose digestion was made, the results of which are shown in figure 7. The increase in the amount of T.C.A. insoluble nitrogen (protein synthesis), as well as cellulose digestion, were measured. From the results obtained, it was concluded that 2 gm. of cellulose substrate would be better suited for further studies.

The data in table 3 give the results obtained on the additive effect of

valeric acid, the amino acids and yeast extract. Two grams of cellulose substrate was used, the digestion being reported in the table as grams of cellulose digested rather than % digestion. The quantity of each material used was considered to be enough to give maximum cellulose digestion. From the results obtained, it appears that the cellulolytic stimulation from the individual materials is not significantly different from the stimulation obtained with the various combinations.

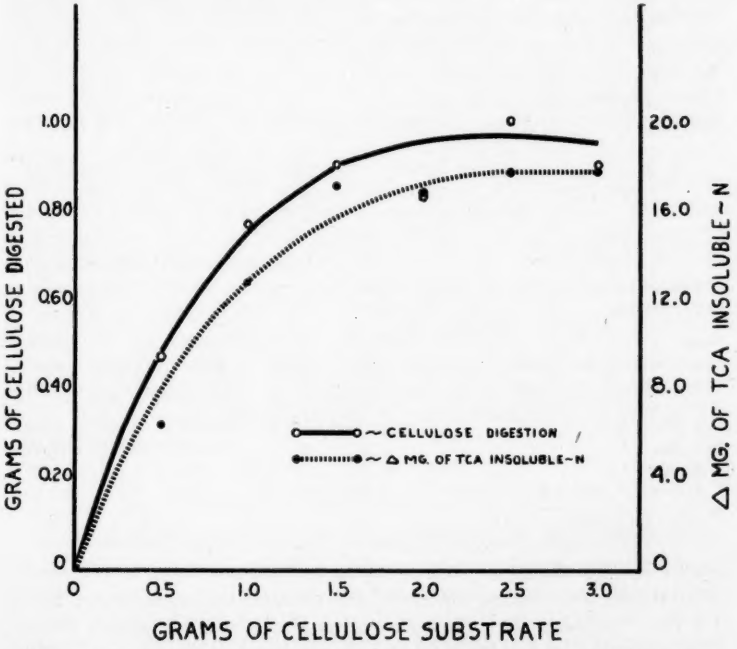

Figure 7. The effect of varying cellulose substrate levels upon cellulose digestion *in vitro*.

To study this additive effect further a standard valeric acid curve was run, with concentrations of valeric acid from 0 to 30 mg. at 5 mg. intervals. A second curve was also run, to which 6 mg. each of valine and proline were added at each level of valeric acid. A slight stimulation of cellulose digestion was observed at the lower valeric acid levels (5 and 10 mgs.), but beyond this no further stimulation due to valine and proline was observed. The cellulolytic activity of valine, proline and the leucines alone and in combination gave essentially the same results with the 2 gm. substrate level as with 1 gm. of cellulose.

Discussion

In the three materials studied (autolyzed yeast, casein hydrolysate and alfalfa extract) the cellulolytic factor activity was consistently found in the same chromatographic fraction (top portion of the phenol chromatogram). Paper chromatographic analysis of these fractions showed that they all contained the amino acids valine, proline, leucine and isoleucine in common. Although in the experiments presented in table 2 on the separation of the active T.P.P.C. of yeast extract the overall activity of the *in vitro* rumen fermentations was less than usual, the data indicate that valine, proline and the leucines are cellulolytically active, while γ-aminobutyric acid is inactive. This idea was substantiated in the experiments utilizing the known amino acids. In the case of yeast extract and casein hydrolysate, the stimulation of cellulose digestion by prepared T.P.P.C. was approxi-

TABLE 3. THE ADDITIVE EFFECT OF THE CELLULOLYTIC FACTOR ACTIVITY OF VALERIC ACID; VALINE, PROLINE AND THE LEUCINES; AND YEAST EXTRACT

Additions to basal medium	Grams cellulose digested				
	Exp. 1	Exp. 2	Exp. 3	Av.	
None	0.323	0.189	0.336	0.283	(.06)[a]
Valeric acid (V.A.), 30 mg.	0.871	0.885	0.804	0.853	(.03)
Amno acids (A.A.), 8 mg. each	0.831	0.764	0.871	0.822	(.04)
Yeast extract (Y.E.), 250 mg.	0.845	0.778	1.005	0.876	(.09)
V.A. plus A.A.	0.992	0.791	0.952	0.912	(.08)
V.A. plus Y.E.	0.845	0.751	1.112	0.903	(.14)
A.A. plus Y.E.	0.912	1.058	1.112	1.027	(.08)
V.A. plus A.A. plus Y.E.	0.898	1.005	1.153	1.019	(.09)

[a] Mean and average deviation.

mately equal to the stimulation of the natural product. With alfalfa extract the fractions obtained by Dowex–50 and charcoal treatment as well as the T.P.P.C., exceeded the cellulolytic activity of the crude alfalfa extract. The possibility of an inhibiting substance present in the crude alfalfa extract which is removed by the purification procedures was suggested by this latter observation. From the results of these experiments it is proposed that the cellulolytic factor activity of yeast extract, casein hydrolysate and alfalfa extract is due to their content of the amino acids valine, proline, leucine and isoleucine. Any material which contains these free amino acids or is an *in vivo* ruminal source of them, would thus be a potential cellulolytically active agent assuming no inhibitors were present.

Inhibition of cellulose digestion by higher levels of casein hydrolysate (from 100–150 mg. upward) was observed in our studies with acid hydrolyzed casein. Inhibition was found to be even more pronounced when enzymatically hydrolyzed casein was used as a factor source. Hershberger

(1955) has also reported this observation. An inhibition of cellulose digestion can be observed with an excess of known amino acids, and has even been observed with higher concentrations of the cellulolytically active amino acids. Hershberger *et al.* (1956) have reported the inhibition of cellulose digestion by the amino acid, alanine. Hall *et al.* (1954) have reported that partial hydrolysates (acid, alkali and enzymatic) of casein are cellulolytically active, while complete hydrolysis resulted in the loss of this activity. Our observations suggest that this loss of activity upon complete hydrolysis may be caused by an excess of liberated amino acids. The partial hydrolysates were possibly found to be active because of the valine, proline and the leucines being released from casein, and the total concentration of the liberated amino acids not yet at the inhibitory level.

The possible mechanisms of the metabolism of valine, proline and the leucines in the *in vitro* rumen fermentation are now being investigated. El-Shazly (1952) reported that proline is converted to δ-amino-n-valeric acid in the *in vitro* rumen fermentation, and postulated that valine and leucine are changed to branched chain 4 and 5 carbon volatile fatty acids. The cellulolytic factor activity of these branched chain C_4 and C_5 volatile fatty acids has been reported previously by Bentley *et al.* (1955). In this present study, δ-amino-n-valeric acid was found to exhibit an additive effect in cellulolytic stimulation, when tested with valine or the leucines. This of course, is similar to the action of proline mentioned earlier.

Since the completion of this work, an article by MacLeod and Murray (1956) has appeared in the literature, reporting that the amino acids valine, leucine and isoleucine stimulate *in vitro* cellulose digestion by washed rumen bacteria.

Summary

The cellulolytic factor activity of autolyzed yeast, casein hydrolysate and alfalfa extract was isolated by Dowex–50 ion exchange resin treatment, charcoal (Darco G-60) treatment and large scale paper chromatography. The assay method used for this cellulolytic activity was the *in vitro* rumen fermentation technique. Factor activity of the materials studied was found to be in the top portion (above R_f 0.65) of the large scale phenol chromatograms. Further fractionation of the eluate from this section (using yeast extract as the starting material), by paper chromatography, indicated that the cellulolytically active factors were valine, proline, leucine and isoleucine. Water extracts of this portion, from all three materials, contained these amino acids in common.

The known amino acids, valine, proline and the leucines were found to be cellulolytically active when used in the *in vitro* rumen fermentation. Proline exerted an additive effect in combination with either valine or the leucines, while valine and the leucines when tested together did not show this effect.

Valeric acid (one of the cellulolytic factors of rumen juice), the amino acids valine, proline and the leucines and yeast extract did not show any appreciable additive effect upon cellulose digestion when tested in various combinations.

Literature Cited

Bentley, O. G., R. R. Johnson, S. Vanecko and C. H. Hunt. 1954. Studies on factors needed by rumen micro-organisms for cellulose digestion *in vitro*. J. Animal Sci. 13:581.

Bentley, O., A. Lemkuhl, R. R. Johnson, T. V. Hershberger, and A. L. Moxon. 1954a. The "Cellulolytic factor" activity of certain short chained fatty acids. J. Am. Chem. Soc. 76:5000.

Bentley, O., R. R. Johnson, T. V. Hershberger, J. H. Cline and A. L. Moxon. 1955. Cellulolytic-factor activity of certain short-chain fatty acids for rumen micro-organisms *in vitro*. J. Nutr. 57:389.

Block, R. J., E. L. Durrum and G. Zweig. *A Manual of Paper Chromatography and Paper Electrophoresis*. Academic Press Inc., New York. 1955.

Bryant, M. P. and R. N. Doetsch. 1955. Factors necessary for the growth of *Bacteroides succinogenes* in the volatile acid fraction of rumen fluid. J. Dairy Sci. 38:340.

Burroughs, W., N. A. Frank, P. Gerlaugh and R. M. Bethke. 1950. Preliminary observations upon factors influencing cellulose digestion by rumen micro-organisms. J. Nutr. 40:9.

Burroughs, W., H. G. Headley, R. M. Bethke and P. Gerlaugh. 1950a. Cellulose digestion in good and poor quality roughages using an artificial rumen. J. Animal Sci. 9:513.

Burroughs, W., J. Long, P. Gerlaugh and R. M. Bethke. 1950b. Cellulose digestion by rumen micro-organisms as influenced by cereal grains and protein-rich feeds commonly fed to cattle using an artificial rumen. J. Animal Sci. 9:523.

Doetsch, R. N., R. Q. Robinson and J. C. Shaw. 1952. Technique employed in cultural investigations of the bacteriology of bovine rumen contents. J. Animal Sci. 11:536.

El-Shazly, K. 1952. Degradation of protein in the rumen of the sheep 2. The action of rumen micro-organsms on amino acids. Biochem. J. 51:647.

Hall, G., E. W. Cheng, W. H. Hale and W. Burroughs. 1954. Chemical and enzymatic preparations of protein hydrolysates stimulatory to cellulose digestion by rumen micro-organisms. J. Animal Sci. 13:985.

Hall, G., E. W. Cheng, W. H. Hale and W. Burroughs. 1955. Properties of unidentified rumen cellulolytic factors in certain protein hydrolysates and their influence on live-weight gains in lambs. J. Animal Sci. 14:1205.

Hershberger, T. V. 1955. Studies on the utilization of non-protein nitrogen by rumen micro-organisms *in vitro*. Ph.D. Dissertation, The Ohio State University, Columbus, Ohio.

Hershberger, T. V., O. G. Bentley, J. H. Cline and W. J. Tyznik. 1956. Formation of short-chain fatty acids from cellulose, starch and metalolic intermediates by ovine and bovine rumen micro-organisms. J. Agr. Food Chem. 4:952.

MacLeod, R. A. and J. F. Murray. 1956. Some factors affecting cellulose digestion by rumen micro-organisms *in vitro*. J. Nutr. 60:245.

McNeill, J. J., R. N. Doetsch and J. C. Shaw. 1954. Some nutritional requirements of bovine rumen bacteria. J. Dairy Sci. 37:81.

Ruf, E. W., W. H. Hale and Wise Burroughs. 1953. Observations upon an unidentified factor in feedstuffs stimulatory to cellulose digestion in the rumen and improved live-weight gains in lambs. J. Animal Sci. 12:731.

FEEDLOT BLOAT STUDIES. II. PHYSICAL FACTORS INVOLVED IN THE ETIOLOGY OF FROTHY BLOAT [1,2]

DON R. JACOBSON, IVAN L. LINDAHL,[3] J. J. MCNEILL, J. C. SHAW, R. N. DOETSCH AND R. E. DAVIS [3]

University of Maryland [4] *and U. S. Department of Agriculture*

BLOAT has been classified as being of the free gaseous and frothy types depending upon the physical nature of the rumen contents at the time the animals bloat. In the frothy type, a large portion of the rumen gas is trapped within the rumen contents (Kick *et al.*, 1937; Olson, 1944; Quin, 1943; Smith *et al.*, 1953; Weiss, 1953; Johns, 1954; Hungate *et al.*, 1955), whereas in the free gaseous type, a major portion of the rumen gas is in the so-called "free form" (Cole *et al.*, 1945).

Quin (1943) postulated that saponin increases the surface tension of the rumen contents and contributes to the development of frothy bloat whereas Weiss (1953) reported that "the formation of froth is dependent on the consistency of the ruminal ingesta which in turn is influenced by reflex salivary secretion."

Since legume pasture and feedlot bloat have many factors in common, it appeared that a greater understanding of feedlot bloat might give considerable insight into the etiology of both. Included herein are the results of one phase of intensive investigations on rumen microbic activity and frothy feedlot bloat. These studies have dealt with the concept of stable froth formation dry matter, consistency index, liquid phase, and percent of encapsulated bacteria in the rumen contents. A brief report of these results appeared elsewhere (Jacobson and Lindahl, 1955, 1955a).

Experimental Plan

An experiment of 16 weeks duration was designed to ascertain and evaluate any physical changes in the rumen contents of cattle prior to, and after being placed on a bloat-producing diet. In addition to the measurement of a number of physical factors, the degree of encapsulation of the ruminal bacteria also was determined.

The four cattle (steers and heifers, 800 to 1000 lb. in weight) reported on in this paper, were maintained on a diet of mixed grass hay and corn

Scientific Article No. A585, Contribution No. 2749 of the Maryland Agricultural Experiment Station.

[1] Taken from a thesis presented by Don R. Jacobson to the Graduate School, University of Maryland, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Dairy Husbandry, University of Maryland.

[2] This work was supported in part by ADPRB, ARS, USDA.

[3] Animal and Poultry Husbandry Research Branch, ARS, USDA, Beltsville, Md.

[4] Bacteriology and Dairy Departments, Maryland Agricultural Experiment Station, College Park.

silage fed ad libitum for eight weeks prior to the beginning of the experiment. While the animals were still on this diet, the first rumen samples were taken to establish the pre-experimental norm. The time at the first sampling was designated as week 0. After these samples were taken the animals were placed on a daily diet of 6 lb. of bloat-producing mixture and 5 lb. of the same lot of mixed grass hay as was fed previously. The bloat-producing mix consisted of 61% barley, 16% soybean oil meal, 1% NaCl and 22% alfalfa meal. The animals were maintained on this diet for four weeks and ruminal samples were obtained again. At the beginning of week 5 the hay was gradually removed from the diet and the bloat-producing mix was increased from 6 to 14 lb. daily over a 10-day changeover period. The animals were maintained on 14 lb. of the bloat-producing diet for the remainder of the experiment. Rumen samples were taken at the end of week 6, or just 4 days following complete changeover to the bloat-producing diet. Thereafter, rumen samples were obtained weekly for seven consecutive weeks (week 7 through 13) and again three weeks later (week 16).

Following the 16-week experiment, a limited number of rumen samples were obtained from the same animals to determine the effect of some materials on the rate of total and stable froth formation in vitro.

Preceding the above experiment a number of observations were made on rumen contents of six animals in a preliminary experiment and on bloated and control sheep and cattle on some seven different diets. Some of these data also are reported.

The feeding and management of the animals and the methods used in rating the severity of the bloat (bloat index) were described in a prior publication (Lindahl *et al.*, 1957). The animals were fed and cared for at the Agricultural Research Center, Beltsville, Md.

Rumen samples were obtained via the esophagus using a standard Colorado rumen tube (outside diameter 1⅜ in., inside diameter 1 in., length 10 ft.) without the aid of a vacuum pump. When the tube was employed, a back and forth action was found to be desirable in obtaining large quantities of rumen contents from different areas of the rumen, thus giving a more representative sample. This technique was employed in sampling all animals not fistulated and was used successfully in taking over 200 rumen samples of approximately 2 to 4 liters each.

Approximately 200 gm. of rumen contents were used in each of the determinations for dry matter. The rumen contents were weighed into an evaporating dish and the volume reduced to about one-fourth on a steam bath. The sample was then dried in an air oven at 105° C. for 24 hours. A final weight was taken and the percent dry matter calculated.

The consistency index (CI) was determined according to the method given by Weiss (1953).

The "ingesta volume increase" (IVI) is a measure, developed in this study, to assign values to the increase in total froth formation in rumen contents in a given period of time. An aliquot (200 ml.) of freshly drawn

rumen contents in a 500 ml. graduated cylinder, was placed into a constant temperature water bath (39° C.) for one hour. The increase in volume was recorded in percent.

The stable "ingesta volume increase" (Stable IVI) was obtained, following the one hour incubation period, by stirring the material in the graduated cylinders 12 times around the full depth of the cylinder with a glass stirring rod. This represents an attempt to measure the relative stability of the total froth produced. The resultant volume was recorded as percent increase or decrease over the original volume prior to incubation and termed stable IVI.

The percent of the original volume of rumen contents which separated into the lower portion of the graduated cylinders (liquid material) in a period of 1 hour was recorded as the percent liquid phase.

In the determination of the percent encapsulated bacteria the strained rumen contents were centrifuged at 771 x G for 10 minutes. The supernatant was then diluted with half strength sterile skim milk to 1 to 400, or to a suitable dilution which presented approximately 20 microorganisms in the microscope field (X970). One hundred ml. of this dilution was spread over one square cm. of slide area and air dried. It was fixed by dipping in 95% ethyl alcohol for one minute and again air dried. Finally, it was stained with Loeffler's alkaline methylene blue solution for one minute and air dried. The encapsulated microorganisms appeared as clear areas surrounding the cells, in contrast to the blue background. A total of approximately 100 microorganisms in five fields was counted, and the number of encapsulated microorganisms also was counted. The percent of microorganisms encapsulated was calculated from these data.

Results

Development of the Concept of Stable Froth Formation in the Rumen and Reticulum. During preliminary investigations it was observed that rumen material freshly drawn from animals on the bloat-producing diet increased in volume markedly when allowed to stand at room temperature. The rumen contents were of a frothy nature upon removal from the rumen and the increase in volume of the rumen contents was considered to be due to additional froth formation. Since the additional froth produced in one hour was equal to the original volume of the rumen contents, it appeared that the observation might have considerable significance in the etiology of bloat. Readings of the increase in volume of several samples were taken at 10-minute intervals over a 3-hour period. The IVI reading obtained at one hour appeared to be the most reliable for comparing large numbers of rumen samples.

It was observed in some cases that the total froth formed in one hour consisted of large bubbles (up to an inch in diameter) and was so unstable that it collapsed spontaneously. In other cases, the froth formed consisted

of small bubbles (mostly smaller than one-eighth inch in diameter) and was so stable that vigorous stirring did not appreciably reduce the volume of the samples. It appeared that if formation of large amounts of froth in the rumen was a factor in bloat, the froth formed would have to be sufficiently stable to resist breakdown during rumino-reticular motility. A simple method was developed for measuring this stable IVI and is given in the section on methods.

Physical Measurements in Relation to the Incidence of Bloat. Early in these studies, 16 IVI and CI values of rumen samples were obtained from six animals on the bloat-producing diet at two hours after feeding. Six of these samples were obtained from animals that were bloated the morning of sampling. There was no significant difference between either the CI or the IVI of samples obtained from bloated vs. nonbloated animals. After the same animals had been on a new diet (8 lb. of the bloat-producing mixture and 15 lb. of alfalfa hay daily) for two weeks, an additional 13 IVI and CI values were obtained. The IVI value for the bloat-producing diet was 94 with a range of 50 to 140 and for the 2:1 hay-concentrate diet it was 67 with a range of 38 to 93. The difference between these two diets was statistically significant at the 1% level. The average CI value for the bloat-producing diet was 90 with a range of 38 to 150 and for the 2:1 hay-concentrate the average CI value was 93 with a range of 30 to 150. The difference was not statistically significant.

The average IVI value of five animals on grass pasture was 25 with a range of 2 to 45 whereas the average IVI value of four animals on Ladino and alfalfa pastures was 38 with a range of 0 to 100. One of the animals on alfalfa pasture was slightly bloated at the time of sampling. On corn silage and mixed grass hay ad libitum, the average IVI value of six samples was 36 with a range of 30 to 40.

Averages of the data obtained from the physical measurements taken during the 16-week experiment are given in table 1. The rumen samples were obtained at approximately 4.5 hours after feeding. The total bloat index, for the four animals, is given for a period consisting of 3.5 days prior to and 3.5 days following the samplings.

The only apparent change in the CI values was between weeks 0 and all other weeks. Even though the animals were bloating during weeks 6 through 16, the CI was not consistently high. Neither was there any specific relationship between the animals bloated and not bloated on the sampling days and their respective consistency indices.

The IVI values were quite variable and relatively low as would be expected for rumen samples taken at 4.5 hours after feeding. At week 13, rumen samples were obtained at both two and 4.5 hours after feeding. The average IVI value was 79 for the samples taken at two hours after feeding and was significantly higher than the average of 47 at 4.5 hours after feeding.

The percentage of rumen contents which separated into the liquid phase

TABLE 1. VARIOUS CHARACTERISTICS OF RUMEN CONTENTS IN RELATION TO THE DEVELOPMENT OF FROTHY BLOAT

Week of experiment	0[a]	4[b]	6[c]	7	8	9	10	11	12	13	16
Av. consistency index of samples	14	51	40	34	31	40	62	38	46	38	77
Av. ingesta volume increase of rumen samples	38	68	43	71	50	52	49	49	48	51	31
Av. percent of rument contents which separated out into a liquid phase in 1 hour	47	29	31	21	22	9	12	24	16	5	0
Av. percent of dry matter of rumen samples	—	8.8	10.0	11.0	11.2	10.8	10.1	10.8	9.0	9.4	8.4
Av. percent of bacteria encapsulated	—	—	—	—	—	49.9	51.3	47.8	58.2	60.4	71.3
Total bloat index of the animals during the week of sampling	0	0	6	18	7	15	18	18	31	47	52

[a] Original samples taken when animals had been on corn silage and mixed grass hay ad libitum for 8 weeks.
[b] Samples taken after four weeks on 5 lb. of mixed grass hay and 6 lb. of bloat-producing mixture per day.
[c] Samples taken 4 days after complete changeover to the bloat-producing diet.

decreased markedly with time on the bloat-producing diet. This was not due to differences in water consumption as the water intake of the animals was regulated preceding the sampling of the rumen contents. A highly significant negative correlation of —0.83 was obtained between the average percent liquid phase and the average bloat index.

The average dry matter content of the rumen samples (table 1) was rather constant, ranging from 8.4 to 11.2 over the 16-week period. There was no correlation between dry matter and CI or between dry matter and percentage liquid phase.

The rumen samples of animals that had been on the bloat-producing diet for a period of time not only contained a small liquid phase but also had a slimy consistency. For this reason it was postulated that encapsulated bacteria were present. A technique was developed whereby the encapsulated bacteria could be readily identified and counted. The encapsulated bacteria observed were of various morphological types. The degree of encapsulation gradually increased from the 9th to the 16th week when the average reached 71% encapsulation. The correlation between the average percentage of encapsulation of the microorganisms and the average bloat index during this period was 0.94 and was highly significant ($P<0.01$).

Effect of Glucose, Saponin and Valeric Acid on IVI and Stable IVI of Rumen Contents. A limited number of trials was carried out to determine the effect of glucose with and without saponin on IVI and stable IVI. The results of one such trial, using 200 ml. aliquots of rumen contents from an animal that had been on the bloat-producing diet for 16 weeks is given in table 2 (rumen sample No. 164). From this and other similar experiments it is evident that glucose and saponin can have an effect, which is more than additive, on the production of stable froth. The data from several other trials are included in table 2 to illustrate in a very general way the effect of time after feeding and several additives on total and stable froth formation.

Discussion

Weiss (1953) reported that the formation of froth is dependent on the consistency of the ruminal ingesta, and that the consistency is increased when the rate of salivation is decreased. Furthermore, since reflex salivary secretion is largely stimulated by the presence of coarse material in the forestomach (Clark and Weiss, 1952) the salivary rate would be reduced on young succulent lucerne. However, the results reported herein indicate that the CI is not closely associated with frothy, feedlot bloat. On the other hand, the high IVI values (obtained from rumen samples taken two hours after feeding) were indicative of potential bloating animals. Even more important in the etiology of bloat is the rate of stable froth formation and the factors that affect it. Glucose was added to the rumen contents in vitro to supply the rumen bacteria with a readily available source of energy and a subsequent increase in the fermentation rate. The effect of added

TABLE 2. EFFECT OF ALFALFA SAPONIN, GLUCOSE AND N-VALERIC ACID ON IVI AND STABLE IVI OF 200 ML. RUMEN SAMPLES

Rumen sample no.	Time after feeding	Additives [a]	IVI	Stable IVI
	min.		%	%
162	25	None-control	75	72
		7.5 gm. glucose	120	110
163	—10	None	15	15
		7.5 gm. glucose	125	10
164	240	None	65	2
		7.5 gm. glucose	98	5
		0.1 gm. saponin	65	10
		7.5 gm. glucose+0.1 gm. saponin	115	112
167	60	None	12	12
		7.5 gm. glucose+0.1 gm. saponin	132	102
		7.5 gm. glucose+0.1 ml. n-valeric acid	88	42
		7.5 gm. glucose+0.2 ml. n-valeric acid	105	28
		7.5 gm. glucose+0.3 ml. n-valeric acid	95	25
		7.5 gm. glucose+0.4 ml. n-valeric acid	88	20
		7.5 gm. glucose+1.0 ml. n-valeric acid	87	12
168	30	None	60	—5
		7.5 gm. glucose+0.1 gm. saponin	68	22
		7.5 gm. glucose+0.1 ml. n-valeric acid	60	—8
		7.5 gm. glucose+0.2 ml. n-valeric acid	92	—15
		7.5 gm. glucose+0.4 ml. n-valeric acid	118	18
		7.5 gm. glucose+1.0 ml. n-valeric acid	65	28
169	95	None	35	6
		7.5 gm. glucose	40	15
		7.5 gm. glucose+0.1 gm. saponin	48	20
		7.5 gm. glucose+0.1 gm. saponin+0.1 ml. n-valeric acid	70	28
		7.5 gm. glucose+0.1 ml. n-valeric acid	80	30
		7.5 gm. glucose+0.2 ml. n-valeric acid	98	70
		7.5 gm. glucose+0.5 ml. n-valeric acid	113	72
171	460	None	66	41
		16 gm. bloat-producing mixture	125	12
		16 gm. bloat-producing mixture+0.2 ml. n-valeric acid	152	8
		7.5 gm. glucose	105	95
		7.5 gm. glucose+0.1 gm. saponin	131	115

[a] The alfalfa saponin used in this study was a composite of several saponins found in alfalfa and was isolated by the Western Utilization Research Branch, ARS, USDA, Albany, California.

glucose on the IVI and stable IVI of rumen samples obtained at 30 minutes to two hours after feeding was much less than in the rumen samples obtained at 4 to 8 hours after feeding. The addition of the bloat-producing feed to rumen samples obtained prior to feeding resulted in the production of large amounts of very unstable froth.

The results from adding n-valeric acid were variable, so that its possible importance in stabilizing the froth produced by rumen contents is not too clear from these studies. It has been shown that certain fatty acids and other compounds in aqeous solutions at optimum concentrations produce varying amounts of stable foam (Weisner, 1939). Of the common volatile fatty acids in rumen fluid, n-valeric acid was the only one that was present in sufficient concentration in the rumen contents of bloating animals to be a factor in stable froth formation. Therefore, the stabilizing effect of n-valeric acid on the froth produced would be dependent upon an optimum concentration and the amount to be added in vitro to obtain a maximum effect on stable froth formation should be dependent upon its initial concentration in the rumen fluid. In addition, the optimum concentration may vary with different rumen samples. Therefore, no specific amount of valeric acid, added in vitro, could be expected to produce an increase in stable froth formation in all cases. The nature of the rumen sample may determine whether the addition of valeric acid will have a material effect on stable froth production.

A number of changes was noted to occur in the physical nature of the rumen contents of animals during the onset of frothy bloat even though the animals were maintained on a constant diet in a relatively constant environment. These changes were of a gradual nature as was the development of bloat. Whereas the percentage dry matter of the rumen contents remained about the same, the average percentage liquid phase gradually decreased to zero in a 10-week period when the animals were on the bloat-producing diet.

In these studies the percentage of microorganisms encapsulated reached a high level by the end of the experiment, and were definitely correlated with the bloat index over the period in which they were counted.

It is of interest to note that a paper by Hungate *et al.* (1955) has appeared recently in which it was suggested that slime production by bacteria may be associated with frothy bloat.

The only apparent change in the CI in the present study occurred when the animals were switched from the diet of corn silage and grass hay to the diet composed partially of grass hay and partially of the bloat-producing mixture. A progressive change in the CI did not accompany the gradual increase in the bloat index. There was no correlation between dry matter and CI in 49 comparisons; therefore, the factor(s) responsible for a high CI must be something other than dry matter.

At the completion of the experiment (after 74 days on the bloat diet) the rumen contents were in a seemingly homogenous frothy state with no apparent stratification taking place. The decrease in percentage liquid phase was correlated with the development of bloat in the present study. There was no correlation between the decrease in the percentage liquid phase and percentage dry matter in the rumen contents. This means that the decrease in the percentage of liquid phase was not due to or associated with

an increase in the dry matter content of the rumen samples. In view of these findings, a rumen sample with a small liquid phase must be one having more water "bound" to the solid particles or at least dispersed throughout the sample.

The IVI values were quite variable and relatively low during the 16-week experiment. A number of factors can result in a low IVI of rumen contents of animals with frothy bloat. In the 16-week experiment, the IVI measurements were made approximately 4.5 hours after feeding, except in one case where they were made at two hours. The values obtained on the samples taken at two hours were significantly higher than those taken at 4.5 hours. This is to be expected as gas production reaches a peak shortly after feeding and then declines rapidly in normal animals (Cole *et al.*, 1942). The rate of gas production in the animals in the present study was also rapid, as the animals usually started to bloat before they finished eating and the height of the bloat occurred at approximately one hour after feeding.

Although it definitely appears that the formation of stable froth was the prime factor in the pathogenesis of bloat in the present reported studies, free gas was always found in the upper regions of the rumina of the animals and a considerable portion of this gas could be released by directing a stomach tube toward the posterior dorsal blind sac. A probable explanation of this observation is that the formation of stable froth interferes with eructation in a manner similar to that described by Weiss (1953), i.e., by "overfilling" the rumen to such an extent that the cardia is not cleared of ingesta by rumino-reticular motility. Recent work by Dougherty and Habel (1955) offers an explanation of how the presence of large amounts of stable froth in the rumen and reticulum could completely block eructation. In cinefluorographic studies, they found that the cardial and diaphragmatic sphincters would permit the esophagus to be filled with either gas, liquid or solid material. However, the cranial esophageal sphincter would permit only gas to escape into the pharynx. Therefore, if stable froth should overlap the cardiac orifice and fill the esophagus at the time the caudal sphincters dilate, eructation would be blocked.

Summary

Increases in both the total and stable froth formation were noted following the removal of rumen contents from cattle on bloat-producing diets. In vitro methods for measuring both were developed. The uncomplicated frothy bloat encountered in this study resulted from a rate of production of stable froth in the rumen and reticulum sufficient to block the eructation mechanism, partially or completely. It was found that small amounts of saponin in the presence of glucose could aid greatly in the formation of stable froth.

A method for the determination of encapsulated microorganisms in

rumen contents is described. In an 8-week period a correlation coefficient of 0.94 was obtained between the percentage of encapsulation of micro-organisms and the occurrence of bloat.

Over a 16-week period during which the incidence of bloat on a dry lot diet increased steadily, a highly significant negative correlation was found between the percentage liquid phase of the rumen contents and the incidence of bloat. No correlation was found between the percentage dry matter of the rumen contents and bloat, between the consistency index of the rumen contents and bloat, or between the dry matter and the consistency index of the rumen contents.

Literature Cited

Clark, R. and K. E. Weiss. 1952. Reflex salivation in sheep and goats initiated by mechanical stimulation of the cardiac area of the forestomachs. J. S. African Vet. Med. Assoc. 23:163.

Cole, H. H., C. F. Huffman, Max Kleiver, T. M. Olson and A. F. Schalk. 1945. A review of bloat in ruminants. J. Animal Sci. 4:183.

Cole, H. H., S. W. Mead and Max Kleiber. 1942. Bloat in cattle. Calif. Agr. Exp. Sta. Bul. 662.

Dougherty, R. W. and R. E. Habel. 1955. The cranial esophageal sphincter, its action and its relation to eructation in sheep as determined by cinefluorography. Cornell Vet. 45:459.

Hungate, R. E., D. W. Fletcher, R. W. Dougherty and G. F. Barrentine. 1955. Microbial activity in bovine rumen: Its measurement and relation to bloat. Appl. Microbiol. 3:161.

Jacobson, Don R. and Ivan L. Lindahl. 1955. Ingesta volume increase in rumen contents of cattle on normal and bloat-producing rations. J. Dairy Sci. 38:606 (Abs.).

Jacobson, Don R. and Ivan L. Lindahl. 1955a. Studies on biochemical, physical and bacteriological factors involved in feedlot bloat. Univ. Md. Misc. Pub. 238:9.

Johns, A. T. 1954. Bloat in cattle on red clover. I. New Zealand J. Sci. Tech. 36:289.

Kick, C. H., P. Gerlaugh and A. F. Schalk. 1937. Some factors affecting the length of time whole corn kernels are retained in the rumens of steers. Am. Soc. Animal Prod. Proc. 95.

Lindahl, Ivan L., R. E. Davis, Don R. Jacobson and J. C. Shaw. 1957. Feedlot bloat studies. I. Animal and dietary factors. J. Animal Sci. (In press).

Olson, T. M. 1944. Bloat in dairy cattle. S. Dak. Agr. Sta. Circ. 52.

Quin, J. I. 1943. Studies on the alimentary tract of Merino sheep in South Africa. VIII. The pathogenesis of acute tympanites (Bloat). Onderstepoort J. Vet. Sci. Animal Indus. 18:113.

Smith, C. K., J. R. Brunner, C. F. Huffman and C. W. Duncan. 1953. Experimental production of frothy bloat in cattle. J. Animal Sci. 12:932.

Weisner, H. B. 1939. Colloid Chemistry. John Wiley and Sons. New York.

Weiss, K. E. 1953. The significance of reflex salivation in relation to froth formation and acute bloat in ruminants. Onderstepoort J. Vet. Res. 26:241.

THE CAROTENE AND VITAMIN A CONTENT OF PLASMA AND LIVER OF RANGE HEREFORD COWS AND THEIR CALVES IN THE NORTHERN GREAT BASIN [1]

R. R. WHEELER,[2] P. H. WESWIG,[3] W. F. BRANNON,[4]
F. E. HUBBERT, JR.[5] AND W. A. SAWYER [2]

Squaw Butte-Harney Range and Livestock Experiment Station [2]

ONLY limited studies have been reported with respect to the carotene and vitamin A status of range cattle in the sagebrush-bunchgrass region of the Northern Great Basin. Hubbert *et al.* (1953) reported the influence of the age of meadow hay on carotene and vitamin A values of the plasma of range cows. The results indicated the necessity for controlled feeding experiments. Therefore, a three-year study was carried out on the response of Hereford cows and their calves to four levels of carotene intake, and a one-year study on blood and hepatic levels of carotene and vitamin A for cows and calves on a "normal" regime.

Experimental Procedure

This experiment involved four winter rations which provided daily carotene intakes of 1.5, 5, 15 and 25 mg. per 100 lb. of body weight, groups I through IV, respectively. Sixteen mature Hereford cows were selected from the Station herd in 1952 and assigned randomly to the four rations (four cows per group). These cows remained on the same ration during the subsequent three winter feeding periods with the exception of a few necessary replacements. (Twelve cows remained in the test groups throughout the three years.)

All cows were fed individually and received a basic ration of bleached native flood-meadow hay, ad libitum, and 3.0 lb. of barley daily. Vitamin supplementation was provided in groups II, III, and IV by adding a beta-carotene rich oil to the barley. This oil contained 7.9 mg. carotene per gram of oil.

The hay was about 90% rushes (*Juncus spp.*) and sedges (*Carex spp.*) and 10% grasses and forbs. The carotene content of the bleached hay was 1.3, 2.4 and 1.5 ppm and the crude protein content was 7.0, 8.0 and 8.1% in 1953, 1954 and 1955, respectively.

[1] Approved for publication as technical paper 999 by the Director of Oregon Agricultural Experiment Station.

[2] Burns, Oregon. Jointly operated by the Agricultural Research Service, United States Department of Agriculture and Oregon Agricultural Experiment Station. Oregon State College, Corvallis.

[3] Department of Agricultural Chemistry, Corvallis.

[4] Formerly Animal Husbandman, Squaw Butte Station, present address: Department of Animal Husbandry, Cornell University, Ithaca, N. Y.

[5] Formerly Animal Husbandman, Squaw Butte Station, present address: Department of Animal Husbandry, Iowa State College, Ames.

The winter experimental feeding periods were initiated in December and terminated in early May of each year. The cows were then moved, with the main herd, to the Squaw Butte unit of the Station to graze sagebrush-bunchgrass range. This type of range provides only two to three months of green grass. The main forage producing species included bluebunch wheatgrass (*Agropyron spicatum*), Idaho fescue (*Festuca idahoensis*), thurber needlegrass (*Stipa thurberiana*), June grass (*Koeleria cristata*) and squirreltail (*Sitanion hystrix*).

In September all cows and calves were moved to the meadow unit of the Station where they grazed flood-meadow aftermath until December. Very little regrowth occurs on these meadows following haying.

To investigate depletion rates of hepatic vitamin A storage, cows on the two low-carotene levels were kept on their respective winter rations throughout the summer and fall of 1955. Because of insufficient bleached hay, barley straw was substituted beginning October 13. Five of the cows were released from the experimental feeding in November and the remaining three in December, after approximately one year on the basal ration. Calves from those two groups of cows received bleached hay, *ad libitum*, and one pound of barley with no additional carotene or vitamin A available from May 12 to July 7. They were then allowed to graze meadows the remainder of the summer.

In May, 1955, six additional cows and their calves were selected from the main herd for an investigation of carotene and vitamin A values of plasma and liver. The cows were fed fair quality meadow hay for approximately five months during the winter. Otherwise the yearly management schedule was the same as the experimental group.

Blood samples were collected from the cows prior to the winter feeding period, before parturition, at the termination of the winter period each year, and at various times during the summer and fall. Blood samples were collected from calves at approximately 28 days of age. In 1955 liver biopsy samples were collected from the cows by the technique of Bone (1954). The liver samples were obtained through an incision between the eleventh and twelfth ribs about 25 to 30 cm. ventrolateral to the junction of the spine and ribs. Several animals posted soon after liver biopsy all showed incision cites in the right lobe.

The analytical methods used have been previously described by Byers *et al.* (1955).

Results and Discussion

Experimental Cows and Calves

Plasma Carotene and Vitamin A. The average plasma carotene and vitamin A values of cows and of their calves at approximately 28 days of age are presented in table 1.

The plasma carotene and vitamin A levels during the three-year study

TABLE 1. AVERAGE PLASMA CAROTENE AND VITAMIN A CONTENT OF COWS AND THEIR CALVES DURING THE EXPERIMENTAL FEEDING PERIODS OF 1953, 1954 AND 1955 (mcg. per ml.)

Blood plasma component	Group No.	1953			1954			1955			Av. 3 yr.	
		Initial 11/19/52	Mid. 2/12/53	Final 5/19/53	Initial 11/12/53	Mid. 2/3/54	Final 5/1/54	Initial 11/16/54	Mid. 2/15/55	Final 4/27/55	Initial	Final
						Cows						
Carotene	I	1.14	0.35	0.31	1.44	0.25	0.18	1.31	0.31	0.30	1.31	0.26
	II	1.17	0.64	0.97	1.76	0.18	0.42	1.07	0.77	0.67	1.33	0.74
	III	1.31	1.75	2.34	1.85	0.29	1.34	1.62	2.00	2.44	1.59	2.04
	IV	0.83	2.15	2.62	1.37	0.87	1.80	0.95	2.68	2.91	1.05	2.44
Vitamin A	I	0.33	0.25	0.15	0.24	0.22	0.12	0.35	0.28	0.22	0.30	0.16
	II	0.35	0.29	0.17	0.26	0.21	0.18	0.39	0.36	0.28	0.33	0.21
	III	0.32	0.32	0.19	0.22	0.20	0.19	0.40	0.38	0.35	0.31	0.24
	IV	0.29	0.22	0.15	0.23	0.23	0.14	0.37	0.38	0.29	0.30	0.19
						Calves						
Carotene	I			0.03			0.01			0.04		0.03
	II			0.02			0.06			0.03		0.04
	III			0.05			0.06			0.10		0.07
	IV			0.10			0.04			0.10		0.08
Vitamin A	I			0.03			0.02			0.05		0.03
	II			0.05			0.07			0.10		0.07
	III			0.10			0.12			0.14		0.12
	IV			0.11			0.06			0.14		0.10

are lower than the values reported by Watkins *et al.* (1950) for a similar period, but are in close agreement with the five-months prepartum levels obtained by Baker *et al.* (1954).

Highly significant differences in blood plasma carotene and vitamin A of cows at the terminal bleeding of the experimental period were demonstrated among the treatment groups all three years. The analysis of variance also showed a highly significant difference among years in both initial and terminal values of plasma carotene and vitamin A. The higher initial plasma carotene values in 1954 can lragely be attributed to the 1953 summer, which had a large amount of precipitation during the months of May and June and provided a longer grazing season on green forage. The

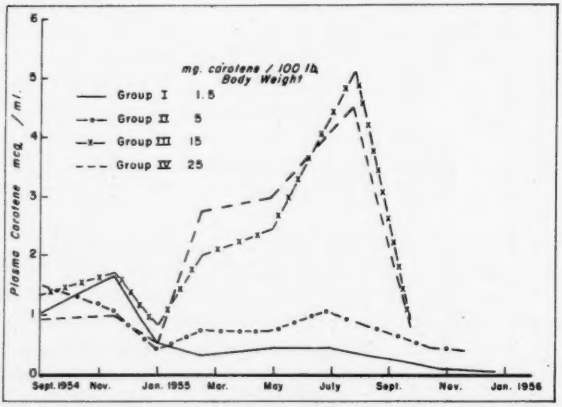

Figure 1. Average values of plasma carotene of cows on 4 levels of carotene intake.

reason for the lower initial plasma vitamin A values in 1954 cannot be explained. Cows in 1954 showed the lowest terminal plasma carotene and vitamin A values of the three-year study.

The plasma carotene of 28-day-old calves corresponded in relative magnitude to the rates of carotene intake of their dams. A similar trend is noted in plasma vitamin A, but it is not as clearly defined. Plasma vitamin A values of calves of groups I and II (table 1) were below the 10 mcg. per 100 ml. level reported by Boyer *et al.* (1942) who stated that plasma vitamin A levels of 7–8 mcg. % were borderline and values below this were inadequate for dairy calves.

Plasma carotene and vitamin A values obtained during July and September on the range showed no distinct differences among groups in 1953 and 1954. The carotene content of the range forage was sufficiently high to nullify the effects of the winter feeding trials.

Figures 1 and 2 demonstrate the effect of carotene intakes on blood

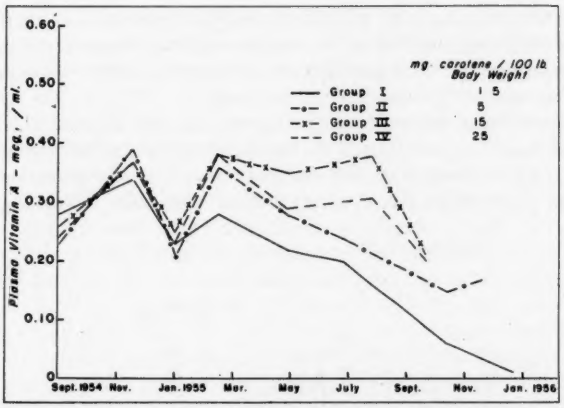

Figure 2. Average values of plasma vitamin A of cows on 4 levels of carotene intake.

plasma carotene and vitamin A of cows, respectively, in 1955. The effects of carotene supplementation were noted within 1.5 months by increased plasma carotene and vitamin A values. Baker *et al.* (1954) did not find an effect of carotene supplementation on plasma carotene until the third month of the experimental period.

There was a gradual decrease in plasma carotene and vitamin A to near zero in cows in group I, which were consuming bleached hay over a period of ten months and straw for two months. The straw contained 0.85 ppm carotene while the hay contained an average of 1.4 ppm.

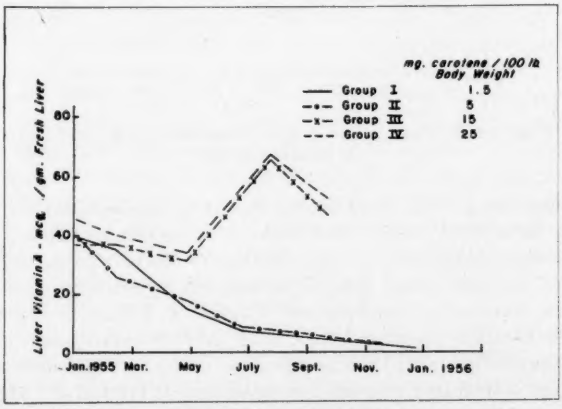

Figure 3. Average values of liver vitamin A of cows on 4 levels of carotene intake.

The cows in group II remained relatively uniform in their plasma carotene until they were taken off the experiment in November. The vitamin A concentration of the plasma decreased somewhat, but was only slightly lower than normal for that time of the year.

Plasma carotene levels of cows in groups III and IV rose in relation to their carotene supplementation during the experimental feeding period and showed a rapid increase during early summer—a direct reflection of their increased carotene intake. A sharp decline was noted when they grazed

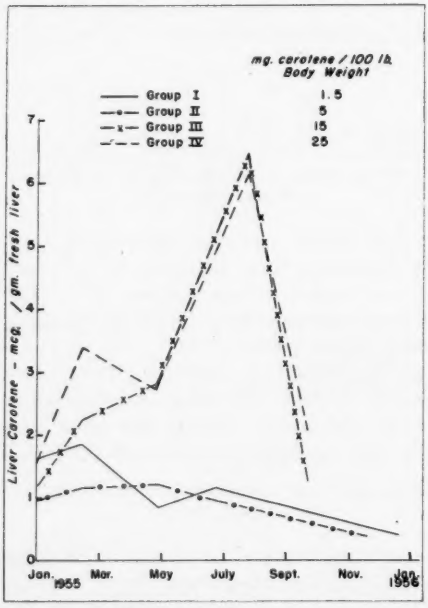

Figure 4. Average values of liver carotene of cows on 4 levels of carotene intake.

cured range forage. The curve is very similar to that reported for Hereford cattle by Long *et al.* (1952) in Oklahoma, when consideration is given to local seasonal conditions. This is in contrast to the twin peak carotene concentration in early spring and fall as reported by Watkins *et al.* (1950).

Hepatic Reserves of Carotene and Vitamin A. Figures 3 and 4 demonstrate the effects of carotene intake upon liver vitamin A and carotene of cows, respectively, in 1955. The effect of carotene supplementation was not evident in increased vitamin A stores in groups III and IV. The gradual decrease during gestation, parturition and lactation even when 25 mg. of carotene per 100 lb. body weight were fed may indicate that the National

Research Council recommendation (1950) of approximately 30 mg. per 100 lb. body weight is too low. Baker *et al.* (1954) found that hepatic stores decreased when 30 mg. of carotene per 100 lb. body weight was fed during lactation. This gradual decrease of vitamin A stores of groups III and IV initially to 28 days postpartum may largely be due to the effect of parturition. Baker *et al.* (1953, 1954) reported losses of hepatic vitamin A stores at parturition. Therefore, as reported by Baker *et al.* (1953), the failure to maintain vitamin A levels in the liver is not necessarily indicative of inadequate carotene intake late in gestation and in early lactation.

The cows in group II had not exhibited symptoms of night blindness at the time of their release from the experimental feeding regime in November. One month later the cows in group I manifested night blindness after almost a year on experimental hay and straw feeding. At that time average hepatic reserves of carotene and vitamin A were 0.42 and 0.82 mcg. per gram, respectively. Hepatic reserves of vitamin A were depleted more rapidly than carotene reserves. This is in agreement with the results of Frey and Jensen (1946).

Liver reserves of carotene and vitamin A of cows in groups III and IV increased when released to green range forage. (Groups I and II were not released to the range in 1955).

Hepatic carotene stores of cows in groups III and IV showed a direct relationship to carotene intake on summer range, peak values when grazing green forage and a rapid drop when grazing cured forage. The relationship of carotene intake to hepatic carotene levels are in agreement with the results reported by Frey and Jensen (1946). No definite relationship existed during the experimental hay feeding period and may have been due to the relatively low levels of carotene supplementation.

Vitamin A Deficiency. No complication accompanied parturition. The calves from cows in all lots were strong and healthy. This would indicate that a carotene intake of 1.5 mg. per 100 lb. live weight accompanied with hepatic vitamin A storage is adequate for gestation and parturition. Davis and Madsen (1941) and Madsen and Davis (1949) found symptoms of deficiency when cows received 2.74 mg. of carotene per 100 lb. body weight when the cows had been previously depleted. It appears that the recommendation of the National Research Council (1950) has a large margin of safety in the allowance of 6 mg. of carotene per 100 lb. of body weight for mature pregnant cows wintered under most range conditions.

Plasma and liver values of carotene and vitamin A are presented in table 2 when calves from cows in groups I and II became night blind and again after grazing green forage for one month.

All calves in groups I and II had exhibited night blindness prior to July 8. Night blindness in both the cows and calves was detected by driving them through a dimly lighted alley in the barn at night where barriers had been placed. On various occasions animals were removed from the range herd and run through the test alley concurrently with the experimental

animals as controls. The control animals had no trouble in dodging the obstacles while the animals affected with night blindness would stumble and fall. The calves developed night blindness rapidly, being able to find their way through the alley one week but not a week later and during subsequent tests.

The plasma and liver levels were very low when night blindness occurred. After one month of grazing green native meadow, plasma and liver levels showed marked increases. Calf No. 547 remained blind after August 9. A necropsy of this animal showed some constriction of the optic foramen. All other calves had regained their night vision acuity within four weeks.

Calf scours were noted in 1953 and 1955 in all groups, but were more prevalent in the calves of groups I and II in 1953. These scours may have

TABLE 2. PLASMA AND LIVER VALUES OF CAROTENE AND VITAMIN A WHEN CALVES WERE DEPLETED OF VITAMIN A AND AGAIN AFTER GRAZING GREEN FORAGE FOR ONE MONTH

Calf No.	Plasma carotene		Plasma vitamin A		Liver carotene		Liver vitamin A	
	7/8/55	8/9/55	7/8/55	8/9/55	7/8/55	8/9/55	7/8/55	8/9/55
	mcg./ml.				mcg./g. fresh			
521	0.13	3.84	0.04	0.12	0.02	1.02	0.53	8.60
522	0.10	3.58	0.02	0.15	0.05	0.53	0.76	14.00
526	0.14	2.42	0.01	0.17	0.06	0.16	0.95	4.63
527	0.10	3.26	0.03	0.16	0.02	1.18	0.21	5.50
547	0.06	2.14	0.05	0.11	0.05	0.76	0.46	6.68
590	0.05	....	0.06	....	0.05	....	0.08	....
591	0.07	3.08	0.05	0.22	0.05	0.89	1.37	10.46
611	0.06	....	0.04	....	0.03	1.57	0.56	10.07
Av.	0.09	3.05	0.04	0.16	0.04	0.87	0.62	8.56

been precipitated by the crowded conditions under which the cows and calves were kept. In 1955 night blindness developed in calves in groups I and II from 13 to 38 days of age. No symptoms were observed in the calves of groups III and IV up to 28 days of age at which time they were released to sagebrush-bunchgrass range.

The fact that vitamin A deficiency occurred in the calves nursing dams receiving 5 mg. of carotene per 100 lb. of live weight demonstrates that this level of feeding is not adequate without other supplementation to support healthy calves. Possibly the calves from cows which received the 15 and 25 mg. levels of carotene would have developed deficiency symptoms also had they been held in the dry lot for an extended period of time. Under most range conditions the calves will have access to hay or range within a few weeks after birth.

Field Cows and Calves. Plasma and liver values of carotene and vitamin

A of the cows and their calves from the main herd are presented in figures 5 and 6, respectively. Plasma carotene and vitamin A and liver carotene values of cows and their calves reflect increased carotene intake on green

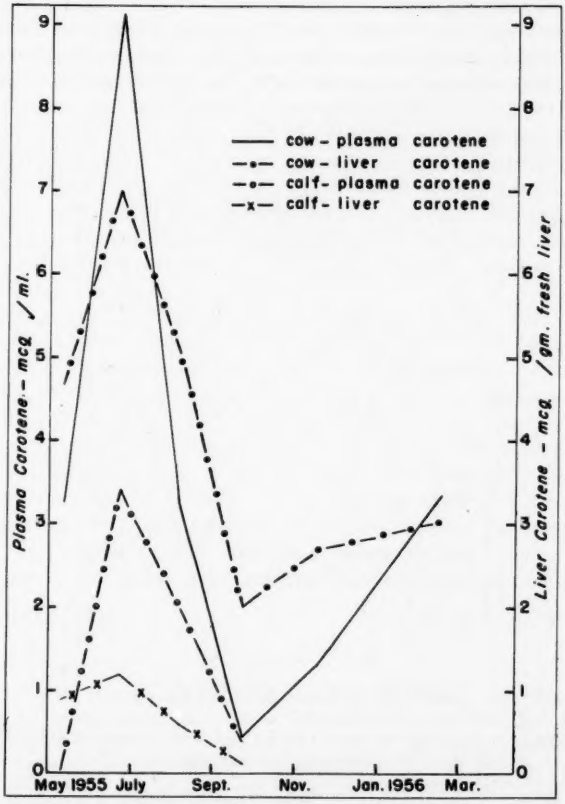

Figure 5. Average values of plasma and liver carotene of field cows and their calves.

spring and early summer forage, and decreased carotene intake on cured forage in the late summer and fall.

The peak of plasma carotene and vitamin A and liver carotene was reached in June, but the peak in hepatic vitamin A levels was not reached until August. These data indicate that plasma carotene and vitamin A and liver carotene values are more responsive to carotene intake than are hepatic vitamin A levels.

Correlation of Plasma Carotene and Vitamin A to Liver Vitamin A. Plasma carotene and vitamin A were both significantly correlated with hepatic vitamin A levels when all of the experimental and field cows were used.

The correlation coefficient of plasma carotene to liver vitamin A was 0.34 and plasma vitamin A to liver A was 0.38. Even though these correlations were statistically significant, extreme variability existed among cows. The degree of association of one variable with the other is so low (r^2=11.6% and 14.4%, respectively) that prediction of vitamin A storage in the liver of an individual cow from carotene and vitamin A concentrations of the plasma would be unreliable. This is in agreement with Thomas and Moore (1952) who reported that the use of plasma levels in a survey to estimate intake or storage may possibly lead to erroneous conclusions.

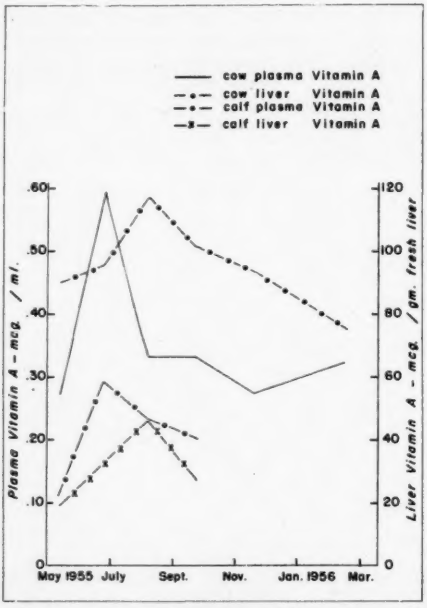

Figure 6. Average values of plasma and liver vitamin A of field cows and their calves.

Summary

Blood plasma and hepatic levels of carotene and vitamin A are reported for cows with carotene intakes of 1.5, 5, 15 and 25 mg. per 100 lb. body weight, respectively by groups I to IV.

Plasma and liver carotene showed a direct relationship to carotene intake during the four-month winter feeding period, and when cows grazed range forage. Hepatic vitamin A reserves were not maintained even at the highest rate of carotene intake during the winter feeding period. Liver vitamin A levels of cows in these high groups increased when they grazed green sagebrush-bunchgrass range during early summer. A continual decrease of the plasma and liver components was noted for cows that were maintained on bleached hay for 10 months and straw for 2 months.

Plasma and liver values of carotene and vitamin A of cows and their calves on the usual range management regime were also investigated. Increased levels were noted when they grazed green range forage, and decreased levels when they grazed cured range forage. Carotene levels increased slightly when they grazed meadow aftermath and continued to increase on green meadow hay. A delay in peak hepatic vitamin A reserves compared to plasma carotene and vitamin A and liver carotene values on summer range was noted.

Cows in group I showed vitamin A deficiency symptoms only after 350 days on the bleached hay and straw ration. In 1955 night blindness was manifested in one-month-old-calves from cows on carotene intakes of 1.5 and 5 mg. per 100 lb. of body weight. The response in plasma and liver levels of these calves to grazing green meadow for one month is presented.

These data indicate that pregnant cows accumulate large stores of vitamin A when grazing spring range which will supply their vitamin A needs during winters even when very low levels of carotene and vitamin A are available in their rations. Vitamin A deficiency may be expected in young calves that are nursing their dams when they are not permitted to consume at least small quantities of hay or other carotene bearing feeds. Such conditions are unusual.

Literature Cited

Baker, F. H., R. MacVicar, L. S. Pope and C. K. Whitehair. 1953. Placental and mammary transfer of vitamin A and carotene by beef cows. Proc. Soc. Expt. Biol. Med. 83:571.

Baker, F. H., L. S. Pope and R. MacVicar. 1954. The effect of vitamin A stores and carotene intake of beef cows on the vitamin A content of the liver and plasma of their calves. J. Animal Sci. 13:802.

Bone, J. F. 1954. A technic for aspiration liver biopsy in dairy cattle. North Am. Veterinarian 35:747.

Boyer, P. D., P. H. Phillips, N. S. Lundquist, C. W. Jensen and S. W. Rupel. 1942. Vitamin A and carotene requirements for the maintenance of adequate blood plasma vitamin A in dairy calves. J. Dairy Sci. 25:433.

Byers, J. H., P. H. Weswig, J. F. Bone and I. R. Jones. 1955. Carotene in the ration of dairy cattle. I. The influence of long periods of sub-optimal carotene intake on the carotene and vitamin A values of the blood, liver, and milk fat of dairy cows. J. Dairy Sci. 38:657.

Davis, R. E. and L. L. Madsen. 1941. Carotene and vitamin A in cattle blood plasma with observations on reproductive performance at restricted levels of carotene intake. J. Nutr. 21:135.

Frey, P. R. and R. Jensen. 1946. Depletion of hepatic reserves of vitamin A and carotene in cattle. J. Nutr. 32:133.

Guilbert, H. R., Paul Gerlaugh and L. L. Madsen. National Research Council. 1950. Recommended nutrient allowances for beef cattle. Washington, D. C.

Hubbert, F. E., Jr., P. H. Weswig, J. R. Haag and W. A. Sawyer. 1953. Influence of age of meadow hay in beef cows' winter rations following summer grazing on sagebrush-bunchgrass range. Proc. of Western Section Am. Soc. Animal Prod. 4: Paper No. XII.

Long, R. A., W. A. VanArsdell, R. MacVicar and O. B. Ross. 1952. Blood composition of normal beef cattle. Okla. Agr. Exp. Sta. Tech. Bul. T–43.

Madsen, L. L. and R. E. Davis. 1949. Carotene requirements of beef cattle for reproduction. J. Animal Sci. 8:625.

Thomas, J. W. and L. A. Moore. 1952. Plasma and storage levels of vitamin A and carotene in relation to intake by calves on different diets. J. Dairy Sci. 35:687.

Watkins, W. E., T. H. Knox and J. W. Benner. 1950. Carotene and vitamin A in the blood plasma of range cows. N. Mex. Agr. Exp. Sta. Bul. 355.

NEWS AND NOTES

The American Society of Animal Production became an Affiliate Member in the American Institute of Biological Sciences on January 1, 1957. Your officers are pleased to announce this affiliation because of the advantages which will result both the American Society of Animal Production and to individual members. Among the services the AIBS is able to afford to its member societies are special placement service, representation with the National Register of Scientific and Technical Personnel, assistance in matters dealing with the Federal fund-granting agencies and with private foundations. It is anticipated that many cooperative activities will be carried out in connection with the AIBS.

A symposium on the nutritional and economic aspects of feed utilization by dairy cows will be held at Michigan State University on May 23–25, 1957. Twenty-five of the leading researchers from many different parts of the United States will participate. All interested in the subject are invited to attend. For detailed information write R. S. Emery, Department of Dairy, or R. G. Wheeler, Department of Agricultural Economics, Michigan State University.

The X International Congress of Genetics will be held at McGill University, Montreal, Canada, August 20–27, 1958. The General Organizing Committee is desirous of having a suitable crest, and requests suggestions be submitted at an early date. Communications should be mailed to J. W. Boyes, Chairman and General Secretary, Department of Genetics, McGill University, Montreal, P.Q., Canada.

Six applied courses in statistical theory and methods, experimental designs and survey designs will be offered by the Department of Statistics at Iowa State College. The first summer session will be from June 18 to July 24; the second, from July 24 to August 30. Students may register for either or both of the six-week sessions. For additional information, write T. A. Bancroft, Department Head and Director, Statistical Laboratory, Iowa State College, Ames.

S. W. Terrill is spending a sabbatical leave from the University of Illinois at the Rowett Research Institute, Bucksburn, Aberdeenshire, Scotland.

W. G. Hoekstra, Assistant Professor of Biochemistry, University of Wisconsin, has been awarded a Merck Senior Postdoctoral Fellowship in the Natural Sciences for the academic year 1957–58. This fellowship, sponsored by Merck and Company, Incorporated, is administered by the National Academy of Sciences—National Research Council. Dr. Hoekstra plans to work on the metabolism and possible site of action of estrogenic compounds through the use of radioactive isotopes and will carry out his research in the Isotope Section of the National Institute for Research in Dairying in Shinfield, England under the direction of R. F. Glascock.

W. F. Williams has recently joined the staff of the Dairy Department of the University of Maryland, where he will conduct research in the Physiology of Milk Secretion and Rumen Function. Dr. Williams was formerly associated with the Dairy Department at the University of Missouri.

W. H. Kyle, Geneticist at the U. S. Sheep Experiment Station, Dubois, Idaho, transferred on February 1 to Purdue University as Assistant National Coordinator of Regional Poultry Breeding Projects.

S. L. Hansard accepted a position as Professor and Animal Husbandman in the Animal Industry Department of Louisiana State University effective February, 1957.

Ralph Boulware joined the Animal Industry Department, Louisiana State University, as Research Associate effective February, 1957.

The joint meetings of the Western Section of the American Society of Animal Production and Western Division of the American Dairy Science Association will be held on the campus of the State College of Washington on July 14–17, 1957.

V16
MAY
XU

INFORMATION FOR CONTRIBUTORS

1. Papers may be submitted reporting work whose ultimate objective is clearly the solution of a livestock production problem and whose contents should be of interest particularly to those dealing with livestock production problems. Review papers on subjects of general interest will also be published at intervals, and will be prepared on invitation by the Editorial Board.

2. All papers will be subject to critical review by the Editorial Board, or others designated by the Editor. Papers needing revision will be returned to authors and should be revised and returned promptly. Papers not suitable for publication will be returned to authors with a statement of reasons for not accepting them.

3. Papers submitted should be based on new and adequate information. The following are guides to style and form:

   (a) Manuscripts must be typed, double-spaced, and the ribbon copy submitted.

   (b) Pages must be numbered. The lines of text must be numbered in the left margin, beginning with one at the top of each page. This does not apply to tabular material.

   (c) Tables should be as few and as simple as is feasible for presentation of the essential data.

   (d) Legend for each figure should be typed on a separate sheet. Photographs should be carefully prepared and labeled. Magnifications of photomicrographs should be indicated on legends. Charts should be prepared in India ink. Typed material on charts is not acceptable. Material that will not reproduce satisfactorily will be returned for revision. For suggestions on preparing illustrative material see the "Style Brief" published by the Wistar Institute Press, Philadelphia. If in doubt, consult the Editor before putting material in finished form.

   (e) The introduction should consist only of a paragraph indicating very briefly the nature of the question asked and the reason for asking it. Reference to relevant literature should be made later in the discussion of the results. Concise presentation of data is desired and there should be evidence of statistical examination wherever obviously useful in the interpretation. Unsupported hypotheses should be avoided. The organization of a manuscript may vary with the nature of the material, but all manuscripts should contain a clear statement of application of the results or their relation to some problem of the livestock industry. Each paper should end with a brief summary in the form of an abstract containing the most pertinent data in the briefest possible form.

   (f) Literature citations must be listed in alphabetical order and should be referred to in the text by author and date, i.e., Castle (1924), or (Castle, 1924). Citations may be referred to by number in review articles where the number of citations is too great to permit convenient use of the above form. "Literature cited" lists should be typed in a style used in a current issue of the JOURNAL.

4. Manuscripts will be edited in the order received, and accepted papers will be published as nearly in this order as feasible.

5. There will be a charge for making engravings in excess of one page per paper and of printing tables in excess of two pages per paper. The author will be asked when his manuscript is accepted to indicate the institution, agency or individual that will assume responsiblity for the charges and for the reprints ordered at that time.

6. Authors will receive galley proof of articles together with a form for supplying an abstract for "Biological Abstracts." *Corrected proof and abstract should be mailed to the Editor within three days after they are received. Failure to do this may result in delay of publication until the next issue.*

7. One author of each manuscript should be a member of the Society. Papers by non-members can be published only after approval by the Executive Committee.

8. A "News and Notes" section will be published in each issue, including Society announcements, changes in positions and other items of professional interest to members. All such items should be sent promptly to the Editor.

9. Abstracts of papers to be presented at the annual meetings of the Society will be published in the November issue. Such abstracts must not exceed 250 words in length, should be typed (double spaced) and the ribbon copy submitted, the lines on the page should be numbered, in the left margin, from top to bottom, and must be in the hands of the Secretary of the Society by August 15, preceding the meeting. These abstracts must be summaries of results and conclusions.

10. Presentation of a paper at the annual meeting does not exclude it from publication in complete form in the JOURNAL. Such papers may be submitted to the Editor and will be given equal consideration with others submitted.

11. All manuscripts and correspondence concerning them should be addressed to the Editor: Dr. J. K. Loosli, Department of Animal Husbandry, Cornell University, Ithaca, New York, or to the publishers: Boyd Printing Company, 372–374 Broadway, Albany 7, New York.

12. All correspondence concerning subscriptions to the JOURNAL OF ANIMAL SCIENCE and other business matters should be addressed to the Business Manager, Dr. H. H. Stonaker, Department of Animal Husbandry, Colorado A&M College, Fort Collins, Colo., or to the publishers, Boyd Printing Company, 372–374 Broadway, Albany 7, New York.

V16
2
M
A
Y
5
XU